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REVISED CLASSIFICATION OF THE BUMBLEBEES—A SYNOPSIS (Hymenoptera: Apidae)[1]

H. E. Milliron[2]

The writer's research on bumblebees began in 1938, continued at frequently interrupted intervals until 1958, but thereafter it has been possible to devote full time to the project. The need for a re-examination of the entire supraspecific classification of bumblebees has been apparent for a long time. However, a study of this particular problem was not seriously undertaken until three years ago in conjunction with taxonomic investigations on the Western Hemisphere species already in progress, and then, with the intention of becoming better informed about the relationships between species of the two hemispheres and their correct nomenclature.

Other workers also are currently engaged in taxonomic and biological studies of these bees, not only in the Western Hemisphere, but in Europe and Asia as well. These efforts are either directly or indirectly related to the results of my own investigations, on which the full report will not appear in print for some months yet to come. In consideration of the amount of work now being done on bumblebees by various individuals, and of the suggestions recently made to me, this preliminary paper has been prepared so that the information on general classification will be available for consideration and use by others. This short contribution cannot include more than the mere essential features of the classification that has been worked out and will be followed in my forthcoming revisional work, which concerns the taxonomy down to the subspecific level. It is, therefore, impossible at this time to go deeply into clarifying the details, amplifying the sets of characteristics for the different groups, or enlarging the substantiating phylogenetic and biological aspects. While complete generic and subgeneric synonymies seem desirable, and are given, the remarks and comments generally are kept to a minimum, and only a very few of the many pertinent works are referred to or cited.

Any taxonomist who works long and intensively on a group should, in due time, adopt or formulate a philosophy which serves as a guide that might enable him to conceive a natural classification, if such is possible. Since the work of Latreille, in 1802, repeated attempts have been made to classify the species of bumblebees, but down to the end of the 19th century most of them have been based on color. Kirby (1802), relying somewhat

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[1] Results presented here are summarized from the current project on a monographic revision of Western Hemisphere bumblebees, which has been aided by grants numbers 1931 and 2640 from the Penrose Fund of the American Philosophical Society, and by grant number G13012 from the National Science Foundation. The writer gratefully acknowledges this assistance.

[2] Since June, 1960, most of the research has been continued at the Carnegie Museum, Pittsburgh, Pennsylvania. The cooperation by this institution and the use of its facilities are deeply appreciated.

on mouthparts, Morawitz (1881-82) on male genitalia, Radoszkowski (1877-78) on mouthparts and later (1883-84, 1888) on male genitalia, were among the notable early exceptions. The system of classifying these species into groups characterized by their genitalia was initiated by Radoszkowski (1883); this was the beginning of a trend toward a natural scheme. Increasing importance was placed upon the male genitalia to and beyond the work of Skorikov (1922); however, other significant morphological features were also used by Franklin (1912-13), Krüger (1917, 1920) and several others.

It has been my contention that too much emphasis and lack of proper interpretation of the male genitalic parts have led to the present wholesale subgeneric division of *Bombus* Latr., which is an unwarranted practice that does not reflect natural continuity or relationships in a phylogenetic system. The classification proposed here is by no means entirely original. Several previous students have made valuable and very influential contributions, *e.g.,* Radoszkowski (1883), Vogt (1911), Sladen (1912), Franklin (1912-13), Krüger (1916, 1917, 1920), and Richards (1927), to name only a few. While it is based to a large extent on the comparative morphology of the male genitalia, it is also supported by other external structures of both male and female. The scope of this study has involved species from both hemispheres, hence the classification is intended to cover the entire subfamily Bombinae.

Briefly, it may be stated that certain features of the male genitalia are more subject to variation between groups than are other parts, and those that appear to be less variable are considered to be more reliable and significant indicators of phylogenetic relationships. Of these parts which in general vary the least, the sagittae are the most important. A comparative study of these alone indicates that the retention of all bumblebees[3] in a single genus results in a very obvious heterogeneous grouping. It further indicates that those species having the sagittal heads straight (or modifications thereof) represent one principal evolutionary line of development, known to me as the Mendax Line (generic status); and that those species having their saggital heads curved inward or mesad (or modifications thereof) represent another principal evolutionary line, known as the Fraternus Line (generic status). Quite a different group of species with flared sagittal heads, trumpet-like in appearance when apposed, comprise a major branch that probably stemmed from the lower Mendax Line, or a progenitor of it; this is referred to as the Terrestris Branch (generic status). The Eurasian Confusus Group, not dealt with here, appears to represent still another major branch similar in derivation and status to that of the Terrestris Branch. The generalized existing members of at least the two principal phylogenetic lines can be rather closely compared morphologically with modern forms of other bees; *e.g., mendax* Gerst. has definite anthophorine affinities and likely arose from an ancestral anthophorine stem, while *fraternus* Sm. exhibits xylocopid affinities and probably arose from the same stem very near where and when the primitive Xylocopini became differ-

[3] This common name, as here used, applies only to the strictly social species.

entiated. The modern representatives of the Terrestris Branch, and those of the Confusus Branch are in reality very different from each other structurally, and their origins are not yet clear; however, it is probable that both are separate off-shoots of the primitive Mendax Line. Suffice to say, the entire true fossil record of bumblebees, or bumblebee-like ancestors is meager and offers little help.

Based on the present distribution of species, the origin of the Fraternus Line is credited to the Western Hemisphere, while that of the Mendax Line is regarded as being in the Eastern Hemisphere. It is believed that, at least during a part of the Tertiary and also likely during parts of the Pleistocene of the Quarternary, there was free passage and frequent exchange of these lines between hemispheres through the Bering area, so that at present both hemispheres possess their descendant representatives. The oldest existing members of the Mendax Line, including the probable Terrestris and Confusus Branches, would appear to have developed in more northern latitudes, or at higher altitudes, but many of the descendant species have also become adapted to an existence at low altitudes and/or at southern latitudes. Contrarily, I believe that when the Fraternus Line became differentiated it then comprised species restricted to more southern latitudes, or to low altitudes, and that only at a later period (late Tertiary—early Quarternary) have their descendants adapted themselves to higher altitudes or to northern latitudes with colder climates.

Past students of bumblebees, with the possible exception of Handlirsch (1888), have been unable to or neglected to devote intensive study to many species from both hemispheres at one and the same time, such as has been done here, possibly because of insufficient material. This, coupled with failure to single out and interpret the lines of evolutionary development referred to above, has been the chief cause for much delay in arriving at a natural taxonomic classification. Of course, the matter of solving the problem was made no easier by the distributional extension of the linear descendants between the two hemispheres in past geological times, without the more primitive forms (e.g., *mendax* and *fraternus*) or their close relatives having likewise extended their ranges. Several past workers, such as Franklin (1912-13), Vogt in Krüger (1917, 1920) and Krüger himself in these cited works, as well as Kruseman (1952) of the present investigators, have indirectly intimated a classification concept suggestive of the present one, but none has fully developed it, perhaps for the reasons already given.

Desired information about certain possible phylogenetically significant features in the behavior patterns of most species of bumblebees is lacking, to say nothing of the inquiline species. A reliable taxonomic classification usually conforms rather closely to a correspondingly acceptable biological classification. The biological arrangement proposed in the commendable work of Sladen (1912, also 1899), especially as modified by Plath (1927) does not, for the most part, conflict with the classification proposed here. However, more of that careful and intensive type of work is needed before the various species behavior patterns concerned with larval nutrition can be properly compared and classified. The taxonomic arrangement then

might have to be altered slightly to better conform to that based on such studies.

EXPLANATION OF SOME SPECIAL TERMINOLOGY

Basal carina—A narrow, curved, glabrous elevated area on the median basal outer surface of the female mandible; not to be confused with the basal parts of the principal carinae (or keels).

Basal vein—The first front-wing vein in the Cresson System directed from the externo-medial toward the front margin; equivalent in the Comstock-Needham System is medio-cubital cross vein, or the proximal part of the median in the system of Ross.

Basitarsus (meso- and meta-)—The basal, or first segment of the respective tarsus.

Discoidal vein—The front-wing vein in the Cresson System extending beyond the basal vein from the externo-medial; equivalent in the Comstock-Needham System is mostly cubitus, and cubitus in the system of Ross.

Holarctic—Refers to the occurrence of a species in at least parts of both the Palaearctic and Nearctic Regions; it is not used here to imply circumpolar distribution.

Incisura lateralis—A prominent emargination on the distal margin of the female mandible near the tip.

Sagitta—One of the innermost pair of male genitalic claspers, or the same as the so-called penis valve; its head is the differentiated distal part.

Sulcus obliquus—A diagonal sharp groove separating one of the principal carinae from the basal area on the lower (or outer) margin of the female mandible.

Supraorbital line—Imaginary transverse line across the vertex connecting the dorsoposterior margins of the compound eyes.

Transverse median vein—The first front-wing vein in the Cresson System directed toward the anal area from the externo-medial; it is cubitus 2 of other systems.

SUBFAMILY *Bombinae*

Characteristics: Moderate to large hairy podilegous bees that differ from other Apidae in having two spurs apically on the hind tibia; malar space prominent, rarely otherwise; first submarginal cell with distinct complete or incomplete transection. *Females* have 12-segmented antennae and distally pointed abdomen composed of six visible terga and six visible sterna. *Males* have 13-segmented antennae and a distally rounded abdomen composed of seven visible terga and six visible sterna; genitalia always with volsella and squama protruding beyond clasper.

The subfamily is divided into two tribes: *Bombini,* or bumblebees, including only the social species; *Psithyrini,* or inquiline bumblebees. The tribes are characterized briefly as follows:

Females

Outer surface of hind tibia, at least in part, flat or concave, devoid of strong hairs except at base and borders (forming a corbicula);

mandibles broad, strongly arched, capable of close overlapping when retracted; labrum rectangular ..... BOMBINI—I

Outer surface of hind tibia convex, hairy (without corbicula); mandibles elongate, tapered distally, weakly arched and incapable of close overlapping when retracted; labrum triangular ..... PSITHYRINI—II

Males

Head short and blocky, usually with strong punctures and coarse pubescence; antennal scape short, one-fourth to one-third as long as flagellum; hind tibia relatively narrow; genitalic vosella and squama membranous ..... PSITHYRINI—II

Head usually longer than wide, triangular to subspherical, with finer punctures and pubescence; antennal scape generally at least one-third as long as flagellum; hind tibia widened distally, its outer surface often lacking hair or with only sparse pubescence; genitalic volsella and squama corneous ..... BOMBINI—I

I. Tribe BOMBINI

The tribe is divided into four genera following the indicated phylogeny. Two of these genera are further divided into subgenera. Supraspecific sectional names,[4] in use heretofore, are not formally recognized although in the past two of these in particular, Odontobombus and Anodontobombus (Krüger, 1917, 1920), have been generally employed for natural groups. To date, a total of sixty generic and sugeneric names have been proposed, each to accommodate one or more species of this tribe; fifteen were originally published as generic names, and forty-five as subgeneric names, although some of the latter also have been used in a generic sense from time to time. The generic and subgeneric names adopted here for each phylogenetic line, or subdivision thereof, have been ascertained according to the recognized genotype designations (Sandhouse, 1943) and the law of priority. While most of these already have been synonymized under the old genus *Bombus* Latr. (Michener, 1944) it becomes necessary to rearrange this synonymy in the light of the present system. Obviously, the concepts covered by many generic and sugeneric names proposed in the past are still retained for species groups, and with few exceptions, the appellations are sufficiently similar to indicate that this is so.

The old heterogeneous *Bombias* Robt. is retained, in part, as a subgenus of the Mendax Line, including two species groups from the Western

[4] Boopobombus Frison (1927) comprises the subgenus *Bombias* Robt. (=*Nevadensibombus* Skor.) of the genus *Megobombus* D.T., and the subgenus *Cullumanobombus* Vogt of the genus *Pyrobombus* D.T.

Odontobombus Krüger (1917) is equivalent to the subgenus *Megabombus* D.T.

Anodontobombus Krüger (1917) is synonymous with the subgenus *Pyrobombus* D.T.

Krüger (1917), in a footnote on page 65 questionably used "Untergattung" in referring to Sulcobombus; however, throughout his work (1917, 1920) it is not clear that he (or Vogt) meant to employ the terms Sulcobombus and Uncobombus in the generic or subgeneric sense. I am presently inclined to regard both as subsectional names, Sulcobombus Krüg. being equivalent to either or both *Confusibombus* Ball and *Megabombus* D.T., while Uncobombus (Vogt) Krüg. corresponds to *Pyrobombus* D.T.

Hemisphere and, of course, the Mendax Group from the Eastern Hemisphere. *Cullumanobombus* Vogt stands in the present classification also as a subgenus (Fraternus Line); however, its limits have been modified or expanded to include considerably more species than have been heretofore assigned to it. To have done otherwise would have meant the recognition of some eleven small subgenera, in place of species groups, in Central and South America, where this segment of the Fraternus Line reaches its maximum diversification.

Key to Genera and Subgenera of Bombini[5]

1. Transverse median vein arising near origin of basal vein, forming inner angle of 90° or less and outer angle of 90° or more; *female*—ocelli large, usually deep-seated, distinctly below supraorbital line (except Cullumanus Group, which has short malar space); meso- and metabasitarsus not spinose distoposteriorly; *male*—ocelli large to medium, usually situated distinctly below supraorbital line; compound eye at least somewhat swollen 2

   Transverse median vein arising distinctly distad of origin of basal vein, forming inner angle of 90° or more and outer angle of 90° or less; *female*—ocelli medium to small, near or at supraorbital line (except Nigrodorsalis Group, which has spinose distoposterior angle on mesobasitarsus); *male*—ocelli small, located at or very near supraorbital line; compound eye not swollen 3

2. Transverse median vein arising at or before origin of basal vein, forming outer angle of 90° or more; *female*—first flagellar segment long, approximately three times longer than distal width, usually longer than second and third combined; malar space moderately long (except in Dentatus Group which lacks sulcus obliquus); mandible without distinct carina on basal area, sulcus obliquus weak or absent; *male*—first flagellar segment usually greatly exceeding third, about equal to second and third combined; compound eyes bulging; sagitta straight (without spinose projection on apical mesal margin) ..... *Megabombus,* subgenus *Bombias* Robt.

   Transverse median vein arising from discoidal vein at most only short distance beyond base, forming outer angle of 90°; *female*—first flagellar segment not more than twice distal width, not exceeding second and third combined; malar space medium to short; mandible usually

[5] Key does not include:

The Eurasian *Confusibombus* Ball (1914), which the writer regards as representing a distinct phylogenetic branch comparable to that of Terrestris; it would run to *Pyrobombus* (Subg. *Pyrobombus*) with which it has no very close affinities whatever.

The Asian (subgenera) *Pressibombus* Frison (1935) and *Tanguticobombus* Pitt. (1909), and the Oriental (genus) *Rufipedobombus* Skor. (1922) have not yet received sufficient study; possibly these, together with the Coccineus Group of the Western Hemisphere, should be combined into a subgenus distinct even from the revised subgenus *Cullumanobombus*.

The Eurasian (genus) *Alpigenobombus* Skor. (1914) is considered not more than a well-defined subgenus of *Pyrobombus* D.T., with synonyms (subgenus) *Mastrucatobombus* Krüg. (1917) and (genus) *Nobilibombus* Skor. (1933) of which the latter's type is here designated as *Bombus nobilis* Skor. (1904).

with carina on basal area, sulcus obliquus and incisura lateralis; *male*—first flagellar segment usually not much longer than third, never equal to second and third combined; sagitta with head hooked mesad (or rarely lanceolate with spine-like projection on apical mesal margin as in Rubicundus Group) ..... *Pyrobombus,* subgenus *Cullumanobombus* Vogt

3. Transverse median vein usually arising a considerable distance from base of discoidal, forming outer angle near 90°; *female*—malar space rather long; mandible usually with distinct sulcus obliquus and carina on basal area, without deep incisura lateralis; mesobasitarsus usually spinose distoposteriorly; *male*—flagellum three or more times longer than scape, median segments often arcuate; sagitta straight, head narrow (divergent or convergent) or variously expanded ..... *Megabombus,* subgenus *Megabombus* D.T.
Transverse median vein arising near base of discoidal; female mandible usually without well-developed carina on basal area, distinct incisura lateralis usually present; otherwise, not like above 4

4. Frontal outline of head round; *female*—tongue short; malar space shorter than distance between (and including) mandibular articulations; mandible with well-developed sulcus obliquus and incisura lateralis; *male*—not markedly dimorphic, with outer hind tibial surface mostly smooth and hairless except at margins; sagitta with head semi-cupped, flared, its dorso-ventral plane broad ..... *Bombus* Latr.
Frontal outline of head triangular or subtriangular, usually at least somewhat higher than wide; *female*—tongue moderately long; malar space equal to or longer than distance between (and including) mandibular articulations; mandible usually without well-developed sulcus obliquus; *male*—markedly dimorphic, most species with at least some hair on outer hind tibial surface; sagitta with head hooked mesad, dorso-ventral plane narrow .... *Pyrobombus,* subgenus *Pyrobombus* D.T.

Genus *BOMBUS* Latr., (1802)

Hist. nat. Crust. et Insect., vol. 3, p. 385.
Type: *Apis terrestris* L., monobasic.

Synonyms:

[*Bremus* Jur., (1801), Erl. Lit.-Ztg., vol. 1, p. 164. Type: *Bombus terrestris* (L.), *(Apis),* desig. by Morice and Durrant, 1915]. Invalidated.

Leucobombus (Bombus) D.T., (1880), Der Naturhist., vol. 2, p. 40. Type: *Bombus terrestris* (L.), desig. by Sandhouse, 1943.

Terrestribombus (Bombus) Vogt, (1911), Sitz.-ber. Ges. naturf. Freunde, Berlin, p. 55. Type: *Bombus terrestris* (L.), desig. by Sandhouse, 1943.

1. TERRESTRIS GROUP: Distribution, Holarctic. Three species in the Western Hempisphere; *terricola* Kby. is one of our typical species.

Genus *MEGABOMBUS* D.T., (1880)

Der Naturhist., vol. 2, p. 40.
Type: (*Bombus ligusticus* Spin.)=*Apis argillacea* Scop., monobasic.

Synonym:

Megalobombus (Bombus) Schultz, (1906), Spolia Hymen., p. 267, monobasic. Emend.

Subgenus *BOMBIAS* Robt., (1903)

Trans. Amer. Ent. Soc. vol. 29, p. 176.
Type: *Bombias auricomus Robt., original desg.* [In part].

Synonyms:

Mendacibombus Skor., (1914), Rev. Russe Ent., vol. 14, p. 125. Type: *Bombus mendax* Gerst., desig. by Sandhouse, 1943.

Nevadensibombus Skor., (1922), Bul. Sta. Règ. Prot. Plantes, Petrograd, vol. 4, p. 149. Type: *Bombus nevadensis* Cress., desig. by Frison, 1927.

1. AURICOMUS GROUP: Distribution, Nearctic. One species, *nevadensis* (Cress.).

2. DENTATUS GROUP: Distribution, Himalayan and Mexican regions. One species in the Western Hemisphere, *haueri* (Handl.). This relic species is closely allied to *dentatus* (Handl.) or to *orichalceus* (Fr.), both of the Himalayan region [*cf. rubicundus* (Sm.) beyond].

Subgenus *MEGABOMBUS* D.T.

Synonyms:

[*Bremus* Pz., (1804), Faun. insect. Germ. init., vol. 8, pl. 20, h. 85. Type: *Apis agrorum* F., desig. by Sandhouse, 1943]. Invalidated.

Alpinobombus Skor., (1922), Rev. Russe Ent., vol. 14, p. 122. Type: *Bombus alpinus* (L.), desig. by Frison, 1927.

Fervidobombus Skor., (1922), Bul. Sta. Règ. Prot. Plantes, Petrograd, vol. 4, p. 153. Type: *Bombus fervidus* (F.), desig. by Frison, 1927.

Mucidobombus Skor., (1922), *Ibid.*, vol. 4, p. 149. Type: *Bombus mucidus* Gerst., desig. by Sandhouse, 1943.

Orientalibombus Rich., (1929), Ann. Mag. Nat. Hist., vol. 3 (ser. 10), p. 378. Type: *Bombus orientalis* Sm., original desig.

Agrobombus (Bombus) Vogt, (1911), Sitz.-ber. Ges. naturf. Freunde, Berlin, p. 52. Type: *Bombus agrorum* (F.), *nec* Schrank, desig. by Sandhouse, 1943.

Hortobombus (Bombus) Vogt, (1911), *Ibid.*, p. 56. Type: *Bombus hortorum* (L.), desig. by Sandhouse, 1943.

Soroeensibombus (Bombus) Vogt, (1911), *Ibid.*, p. 63. Type: *Bombus soroeensis* (F.), monobasic.

Subterraneobombus (Bombus) Vogt, (1911), *Ibid.*, p. 62. Type: *Bombus subterraneus* (L.), desig. by Frison, 1927.

Tricornibombus (Agrobombus) Skor., (1922), Bul. Sta. Règ. Prot. Plantes, Petrograd, vol. 4, p. 151. Type: *Bombus tricornis* Rad., monobasic.

Adventoribombus (Agrobombus) Skor., (1922), *Ibid.*, p. 150. Type: *Agrobombus adventor* Skor., desig. by Sandhouse, 1943.

Laesobombus (Agrobombus) Skor., (1922), *Ibid.*, p. 150. Type: *Bombus laesus* Mor., monobasic.

Exilobombus (Mucidobombus) Skor., (1922), *Ibid.*, p. 150. Type: *Mucidobombus exil (exiln sic!)* Skor. (=*Bombus nymphae* Skor.), monobasic.

Diversobombus (Bombus) Skor., (1914), Rev. Russe Ent., vol. 14, p. 406. Type: *Bombus diversus* Sm., desig. by Sandhouse, 1943.

Kallobombus (Bombus) D.T., (1880), Der Naturhist., vol. 2, p. 40. Type: *Apis soroeensis* F., desig. by Sandhouse, 1943.

Callobombus (Bombus) D.T., (1896), Cat. Hymen., vol. 10, p. 503. Emend.

Chromobombus (Bombus) D.T., (1880), Der Naturhist., vol. 2, p. 40. Type: *Apis muscorum* L., desig. by Sandhouse, 1943.

Rhodobombus (Bombus) D.T. (1880), *Ibid.*, vol. 2. p. 40. Type: *Bombus pomorum* (Pz.), desig. by Sandhouse, 1943.

Pomobombus (Bombus) Krüg., (1917), Ent. Mitt., vol. 6, p. 65. Type: *Bombus pomorum* (Pz.), desig. by Sandhouse, 1943.

Senexibombus (Bremus) Fris., (1930), Treubia, vol. 12, p. 3. Type: *Bombus senex* Voll., original desig.

1. ALPINUS GROUP: Distribution, Holarctic. Four Western Hemisphere species, of which *polaris* (Curt.) (=*arcticus* Kby., *nec* Acerbi) is typical here.

2. SUBTERRANEUS GROUP: Distribution, Holarctic. Two Western Hemisphere species, of which *borealis* (Kby.) is typical here.

3. DUMOUCHELI GROUP: Distribution, Holarctic. Thirteen Western Hemisphere species, of which *fervidus* (F.) is typical.

4. NIGRODORSALIS GROUP: Distribution, Mexico and Central America. Three species, of which *nigrodorsalis* (Fkln.) is typical.

5. MEXICANUS GROUP: Distribution, Mexico, Central and northern South America. Four species are recognized here, of which *mexicanus* (Cress.) is typical.

In this subgenus there are, in addition, several distinct species groups that are restricted to Eurasia.

Genus *PYROBOMBUS* D.T., (1880)

Der Naturhist., vol. 2, p. 40.
Type: *Bombus hypnorum* (L.), monobasic.

Synonym:

Pyrrhobombus (Bombus) D.T., (1882), Ber. naturw.-med. Ver., Innsb., vol. 2, p. 28. Emend.

Subgenus *CULLUMANOBOMBUS* Vogt, (1911)

Sitz.-ber. Ges. naturf. Freunde, Berlin, p. 57.
Type: *Bombus cullumanus* (Kby.), desig. by Frison, 1927.

Synonyms:

Bombias Robt., (1903), Trans. Amer. Ent. Soc., vol. 29, p. 176. [In part].

Volucellobombus Skor., (1922), Bul. Sta. Règ. Prot. Plantes, Petrograd, vol. 4, p. 149. Type: *Bombus volucelloides* Grib., monobasic.

Fraternobombus (Alpigenobombus) Skor., (1922), *Ibid.*, vol. 4, p. 156. Type: *Apathus fraternus* Sm., desig. Frison, 1927.

Funebribombus (Alpigenobombus) Skor., (1922), *Ibid.*, vol. 4, p. 157. Type: *Bombus funebris* Sm., monobasic.

Robustobombus (Alpigenobombus) Skor., (1922), *Ibid.*, vol. 4, p. 157. Type: *Bombus robustus* Sm., desig. by Sandhouse, 1943.

Rubicundobombus (Fervidobombus) Skor., (1922), *Ibid.*, vol. 4, p. 154. Type: *Bombus rubicundus* Sm., desig. by Sandhouse, 1943.

Rufocinctobombus (Bremus) Fris., (1927), Trans. Amer. Ent. Soc., vol. 53, p. 78 (pl. 17, fig. 9). Type: *Bombus rufocinctus* Cress., monobasic.

Separatobombus (Bremus) Fris., (1927), *Ibid.*, vol. 53, p. 64. Type: (*Bombus separatus* Cress.)=*Apis griseocollis* De G., original desig.

Sibiricobombus (Bombus) Vogt, (1911), Sitz.-ber. naturf. Freunde, Berlin, p. 60. Type: *Bombus sibiricus* (F.), desig. by Sandhouse, 1943.

Crotchiibombus (Bombus) Fkln., (1954), Trans. Amer. Ent. Soc., vol. 80, p. 51. Type: *Bombus crotchii* Cress., original desig.

1. FRATERNUS GROUP: Distribution, Nearctic. *Fraternus* (Sm.) is the only species.

2. GRISEOCOLLIS GROUP: Distribution, Nearctic. Comprises two species, of which *griseocollis* (De G.) is typical.

3. CROTCHII GROUP: Distribution, Nearctic. *Crotchii* (Cress.) is the only species.

4. BRACHYCEPHALUS GROUP: Distribution, Mexico and Central America. *Brachycephalus* (Handl.) is the only species.

5. VOLUCELLOIDES GROUP: Distribution, Neotropical. Two species, of which *volucelloides* (Grib.) is typical.

6. ROBUSTUS GROUP: Distribution, Neotropical. Five species are included here, *robustus* (Sm.) being typical.

7. FUNEBRIS GROUP: Distribution, Neotropical. *Funebris* (Sm.) is the only species.

8. RUBICUNDUS GROUP: Distribution, Neotropical. *Rubicundus* (Sm.) is the only species. This relic has pronounced affinities with certain Himalayan species, and warrants further study.

9. HANDLIRSCHI GROUP: Distribution, Neotropical. *Handlirschi* (Fr.) is the only species.

10. CULLUMANUS GROUP: Distribution, Holarctic. The Western Hemisphere has only *rufocinctus* (Cress.) which extends into Mexico. This species is not markedly different from its Eastern Hemisphere relatives.

11. COCCINEUS GROUP: Distribution, Neotropical. Comprises two species, of which *coccineus* (Fr.) is typical.

The Sibiricus Group, which is also placed in this subgenus, is confined to Asia and to the extreme eastern part of Europe.

Subgenus *PYROBOMBUS* D.T.

Synonyms:

Kozlovibombus Skor., (1922), Bul. Sta. Règ. Prot. Plantes, Petrograd, vol. 4, p. 152. Type: *Bombus kozlovi* Skor., desig. by Sandhouse, 1943.

Bombellus, nom. nud., (nec Wild), (1931), Zool. Rec., p. 248. Error.

Pratobombus (Bombus) Vogt, (1911), Sitz-ber. Ges. naturf. Freunde, Berlin, p. 49. Type: *Apis pratorum* L., desig. by Frison, 1927.

Lapidariobombus (Bombus) Vogt, (1911), *Ibid.*, p. 58. Type: *Bombus lapidarius* (L.), desig. by Sandhouse, 1943.

Melanobombus (Bombus) D.T., (1880), Der Naturhist., vol. 2, p. 40. Type: *Bombus lapidarius* (L.), desig. by Sandhouse, 1943.

Thoracobombus (Bombus) D.T., (1880), *Ibid.*, vol. 2, p. 40. Type: *Apis sylvarum* L. desig. by Sandhouse, 1943.

Poecilobombus (Bombus) D.T., (1882), Ber. naturw.-med. Ver., Innsb., vol. 12, p. 23. Type: *Bombus sitkensis* Nyl., desig. by Sandhouse, 1943.

Obertobombus (Bombus) Reinig, (1930), Zeitschr. f. Morph.-Ökol. Tiere, vol. 17, p. 107. Type: *Bombus oberti* Mor., monobasic.

Hypnorobombus (Bombus) Perez, M. Q., (1927), An. Inst. Nac., Valencia, Trab. Lab. Hist. Nat., No. 16, p. 19 (20). Type: *Bombus hypnorum* (L.), monobasic.

Lapponicobombus (Bombus) Perez, M. Q., (1927), *Ibid.*, No. 16, p. 19 (20). Type: *Bombus lapponicus* (F.), present designation (by virtual tautonymy).

1. PRATORUM GROUP: Distribution, Holarctic. Eight known species occur in the Western Hemisphere; *impatiens* (Cress.) is a typical representative.

2. JONELLUS GROUP: Distribution, Holarctic. Four species occur in the Western Hemisphere; *frigidus* (Sm.) is the best known of the typical species.

3. PRATICOLA GROUP: Distribution, Holarctic. Six species occur in the Western Hemisphere; *vagans* (Sm.) is a typical species.

4. LAPPONICUS GROUP: Distribution, Holarctic. The nearly circumpolar *lapponicus* (F.) is typical.

No additional species groups, requiring recognition in this subgenus, are at present known to me. However, a better understanding of certain Eurasian species complexes might result in somewhat expanding the above groups, or even the addition of new ones.

II. Tribe PSITHYRINI

The inquiline bumblebees are regarded as constituting a generically homogeneous group, comprising a tribe separate from the social bumblebees, as already indicated. Nothing has been uncovered in this study to contradict the belief that they are monophyletic. Possibly the group originated as early as the middle of the Tertiary, and evolved very slowly. All species of *Psithyrus* have, however, undergone such remarkable and similar structural modifications consistent with their habit that it is difficult to determine from which branch they came. There is reason to believe that the most primitive species evolved either directly from the Mendax Line, or from a branch thereof possibly very close to the time when either the Confusus or Terrestris Group became differentiated.

Genus *PSITHYRUS* Lep., (1832)

Ann. Soc. Ent., France, vol. 1, p. 373.
Type: *Apis rupestris* F., desig. by Curtis, 1833.

Synonyms:[6]

Apathus Newm., (1835), Ent. Mag., vol. 2, p. 404 (footnote). Type: *Apis rupestris* F., autobasic.

Bremus Kby., (1837), Faun. Bor.-Amer., Pt. 4, p. 272 (footnote). Type: *Apis rupestris* F., present designation.

Bombus Greene, (1860), Ann. Lyc. Nat. Hist. N.Y., vol. 7, p. 169. [In part].

Ashtonipsithyrus (Psithyrus) Fris., (1927), Trans. Amer. Ent. Soc., vol. 53, p. 69. Type: *Psithyrus ashtoni* (Cress.), original desig.

Fernaldaepsithyrus (Psithyrus) Fris., (1927), *Ibid.*, p. 70. Type: *Psithyrus fernaldae* Fkln., monobasic.

Allopsithyrus (Psithyrus) Popov, (1931), Eos, vol. 7, p. 136. Type: *Psithyrus barbutellus* (Kby.), original desig.

Eopsithyrus (Psithyrus) Popov, (1931), *Ibid.*, p. 134. Type: *Psithyrus tibetanus* (Mor.), original desig.

Metapsithyrus (Psithyrus) Popov, (1931), *Ibid.*, p. 135. Type: *Psithyrus campestris* (Pz.), original desig.

Ceratopsithyrus (Psithyrus) Pitt., (1949), *Ibid.*, vol. 25, p. 271. Type: *Psithyrus klapperichi* Pitt., monobasic.

1. VESTALIS GROUP: Distribution, Holarctic. Two species are found in the Western Hemisphere; *suckleyi* (Greene) is a typical representative.

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[6] *Laboriopsithyrus (Psithyrus)* Fris., (1927), Trans. Amer. Ent. Soc., vol. 53, p. 69. Its type species, *(Bombus) Psithyrus laboriosus* (F.), belongs in *Emphoropsis* Ashm. (1899). Perhaps a ruling by the Commission will be necessary.

2. CAMPESTRIS GROUP: Distribution, Holarctic. Eight known species occur in the Western Hemisphere; our *insularis* (Sm.) is typical of the group.

3. QUADRICOLOR GROUP: Distribution, Holarctic. Two subspecies of the circumpolar *quadricolor* Lep. are found in the Western Hemisphere; *fernaldae* Fkln. is typical of one subspecies.

There are several Eurasian species which appear to form two or three species groups not recognized above.

SUMMARY

The revised classification places the bumblebees in the subfamily Bombinae consisting of two tribes, *Bombini* (social species, or bumblebees) and *Psithyrini* (inquiline species, or inquiline bumblebees). The old genus *Bombus* Latr., to which all social species are referable, is divided into four genera, of which *Megabombus* D.T. and *Pyrobombus* D.T. each represents one of the two principal phylogenetic lines (Mendax and Fraternus), and *Bombus* Latr. *(s.s.)* and *Confusibombus* Ball each represents a branch, possibly from the Mendax Line. The old genus *Psithyrus* Lep., which contains all inquiline bumblebees, appears to be a monophyletic and homogeneous group in which it seems unnecessary and undesirable to recognize subgenera.

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A STRANGE *HYDROMETRA* FROM THE MALAY PENINSULA (Heteroptera: Hydrometridae)[1]

HERBERT B. HUNGERFORD AND RYUICHI MATSUDA

Among the specimens of *Hydrometra* sent to the senior author after publication of "The Hydrometridae of the Hungarian National Museum and other studies in the family" by Hungerford and Evans (Ann. Mus. Nat. Hung. 28:31-112, 1934), a female brachypterous specimen taken from Selangor, Malaya, is so unique and puzzling that he wondered if the finger-like projection from the caudal margin of the pronotum could be an abnormality of some described species and has endeavored through the years to obtain more specimens, but without success.

In our recent study we have decided that it is indeed an undescribed species, not a freak of nature, and we are renewing our efforts to secure specimens from the type locality.

Hydrometra aberrans n. sp.
(Figures 1-6)

Size: Length 14.7 mm. (brachypterous female).

Color: Light yellowish brown, with abdominal dorsum a little darker and sides of abdomen with a longitudinal dark band.

Structural characteristics: Ratio of anterior part of head to posterior part of head (AO:PO)::114:40.[2] Length of head in relation to length of thorax (from anterior margin of proacetabula to hind margin of metacetabula)::167:130. Dorsal interocular groove appears deep due to elevated ridge on either side; ventral interocular groove wider and its margins also elevated. Clypeus broad, its sides parallel, distal end as shown in Figure 2,

[1] Contribution number 1090 from the Department of Entomology, The University of Kansas. This study is a by-product of a project supported by a grant from the National Science Foundation.

[2] A unit of measurement is equal to 0.0333 mm.

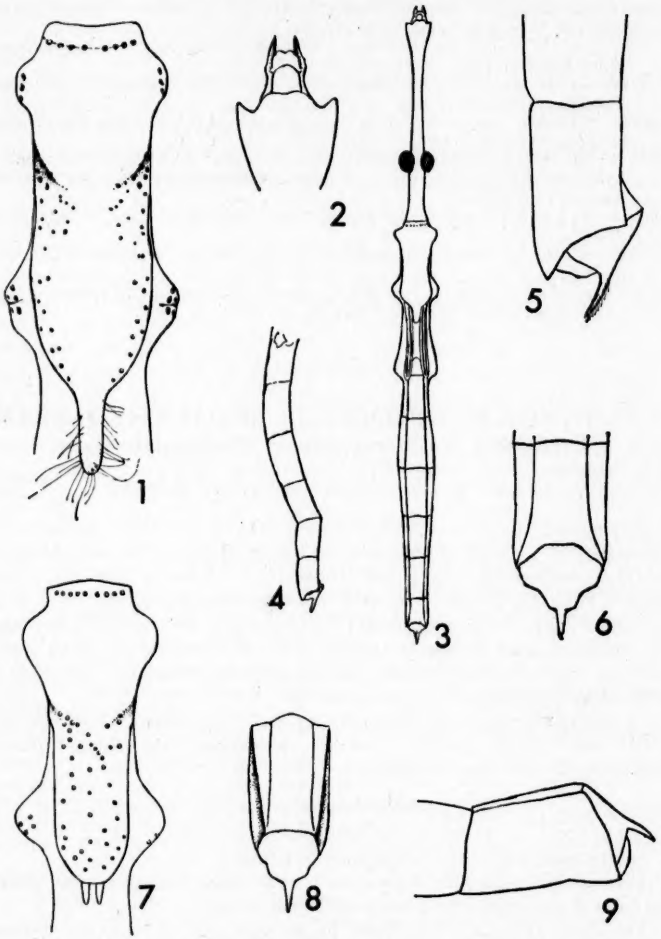

PLATE I

Hydrometra aberrans Hungerford and Matsuda. Fig. 1. Pronotum of brachypterous female showing caudal projection, but omitting greatly reduced wings that are shown in figure 3. Fig. 2. Anterior portion of head showing shape of clypeus. Fig. 3. Dorsal view of species. Fig. 4. Abdomen in lateral view. Fig. 5. Lateral view of terminal abdominal segments. Fig. 6. Dorsal view of terminal abdominal segments.

Hydrometra longicapitis Torre-Bueno. Fig. 7. Pronotum of micropterous female showing greatly reduced wings projecting from beneath caudal margin of pronotum. Fig. 8. Dorsal view of terminal abdominal segments. Fig. 9. Lateral view of terminal abdominal segments.

its median length to its width::15:17. Beak would reach to anterior half of PO. Antennal formula:1st:2nd:3rd:4th::28:57:170(?):85.

Length of pronotum including caudal projection 85 units, of which the projection 17 units (Fig. 1). An encircling row of coarse pits parallel to anterior margin and about 4 units behind it. Several coarse pits on either side of proacetabular cleft. Posterior lobe of pronotum with a number of coarse pits and bearing hairy caudal projection. Meso- and metacetabula with several coarse pits. Reduced wings reaching rear margin of metacetabula and showing some veins. Distance between first and second coxae to that between second and third coxae::40:70.

The relative lengths of leg segments.

	Femur	Tibia	Tarsus
Front leg	180	208	28
Middle leg	210	230	30
Hind leg	258	335	30

In side view, first four abdominal segments forming a curve with its lowest point between second and third segments and rising to end of fourth, where last two segments make a sudden bend and are horizontal. Length of last abdominal tergite to that of the one before it::21:30. Suranal plate obliquely turned downward, its caudal spine less than half of total length of suranal plate.

Comparative notes: This is one of the few species of *Hydrometra* having the head conspicuously longer than the thorax. Comparing it with *Hydrometra longicapitis* Torre-Bueno from Thailand (a male specimen of which was compared with the solitary type specimen of *H. longiscapitis* Torre-Bueno by Dr. R. I. Sailer of the U.S. National Museum and declared to be the same) and from Java, the female of this new species differs from that of the latter species as follows:

1. The pronotum is provided with a conspicuous caudal projection in *H. aberrans*.

2. The body is larger in *H. aberrans* than in *H. longicapitis*.

3. The contour of the abdomen in *H. aberrans* is peculiar as described above.

4. The abdominal tergites are relatively broader in *H. aberrans* than in *H. longicapitis*. For example, the basal width of the last abdominal tergite relative to its length::35:62 and the same of the preceding tergite::45:95 in *H. aberrans*, whereas the ratio of the basal width of the last abdominal tergite to its length::22:65 and the same of the preceding tergite::27:80 in *H. longicapitis*.

The presence of the caudal projection of the pronotum in this new species separates it from all the known species. It may well be a sexually linked character confined to the brachypterous or apterous female. We would expect the male of this new species to show a close relationship to *H. longicapitis*.

Holotype: One brachypterous female taken from a duck pond, 17 miles (from?) Dusun Tua, Selangor, Malay Peninsula, Aug. 26, 1926, Sulaiman *leg.*

EFFECTS OF MALATHION AND PIPERONYL BUTOXIDE COMBINATIONS ON THE RATE OF OXYGEN CONSUMPTION OF HOUSE FLIES[1]

M. T. OUYE,[2] LALLAN RAI,[3] AND C. C. ROAN[3]

The rate of oxygen uptake is a quantitative expression of the total physiological activity of an intact insect. By subjecting the insect to lethal and sublethal doses of toxicants, the effect of the toxicant on oxygen consumption can be observed from treatment to death.

Lord (1950) showed that injection of diethyl-*p*-nitrophenyl thiophosphate (parathion) and hexaethyl tetraphosphate (HETP) caused an increase in oxygen uptake before killing *Tribolium castaneum* Hbst. Harvey and Brown (1951) found that injection of tetraethyl pyrophosphate (TEPP) or parathion into German cockroaches had a marked initial stimulatory effect on the rate of oxygen consumption. They concluded that, under similar experimental conditions, organic phosphates should react similarly.

O'Brien (1956) investigated the respiration of malathion-poisoned roaches and did not find a marked increase in oxygen consumption. The method used was similar to that of Harvey and Brown (1951) except for using *Periplaneta* nymphs instead of *Blattella* adults and propylene glycol instead of corn oil for the solvent.

The present investigation was undertaken to determine the effect of malathion, alone and in combination with piperonyl butoxide, on the rate of oxygen consumption of two strains of house flies, and to correlate mortality with oxygen consumption.

MATERIALS AND METHODS

Two strains of house flies, *Musca domestica* L., were used. The strain designated as KUN (malathion susceptible) has been reared in this laboratory for 10 years with no previous history of insecticidal exposure. The strain designated as CAL (malathion resistant) was acquired from the University of California Citrus Experiment Station at Riverside, and subsequently reared in this laboratory for eight generations before testing. The CAL strain was known to be at least 10 times as resistant to malathion as the KUN strain.

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[2] Now Entomologist, Pink Bollworn Section, Entomology Research Division, United States Department of Agriculture, Brownsville, Texas.

[3] Assistant Professor and Professor, respectively, Department of Entomology, Kansas State University, Manhattan.

These flies were reared in the laboratory in accordance with standard CSMA procedures modified only with reference to the adult diet (Rai *et al.*, 1956). The insecticide-free rearing room was maintained at a temperature of 80°±2° F.

Only four-day old female flies were used. The flies were anaesthetized with carbon dioxide to facilitate handling and sexing. Carbon dioxide anaesthesia was kept at a minimum, well within limits designated by Williams (1946).

The compounds used were malathion and technical piperonyl butoxide. Acetone solutions of piperonyl butoxide and malathion were made at a ratio of 10 parts of piperonyl butoxide to one part malathion. Individual topical applications of 1.35±0.05 microliters were made on the mesosternum of each fly with a micro-device described by Roan and Maeda (1953).

Oxygen consumption at 30° C. was measured by the Warburg method described by Umbreit, Burris, and Stauffer (1949). Sixteen milliliter capacity double sidearm flasks were used. Carbon dioxide produced by the fly was removed by 0.4 milliliter of 20 per cent potassium hydroxide solution which was placed in the center well of the flask with a rolled strip of filter paper. A loose wad of cotton was placed into the opening of the sidearm leading into the body of the reaction flask. The cotton was placed so that a fly could be introduced into the sidearm from the sidearm "vent."

The manometers, flasks, and contents were equilibrated in the temperature of the bath for 20 minutes. The manometer support was placed so that the opening of the unstoppered sidearm was about an eighth of an inch above the water line.

The flies were weighed individually before treatment so that the data could be expressed as cu. mm. 0_2/min./mg. of fly. After treatment, the flies were placed, one per flask, into the unstoppered sidearm which was subsequently stoppered. The manometers were then lowered so that the flasks were completely submerged. The stopcocks were left open for five minutes after introduction of the flies into the sidearm so that the acetone solvent would evaporate from the flask. Tht stopcocks were subsequently closed. Measurements commenced within 10 minutes after treatment. Readings were made every 10 minutes for the first 30 minutes after treatment and every 30 minutes thereafter. In most cases, the experimental periods were 660 minutes. Eight manometers and two thermobarometers were used with each experimental period. In order to minimize physical activity of the flies the shaking apparatus was not used.

Previous preliminary experiments showed that there were no differences in respiration resulting from acetone treatments or piperonyl butoxide treatments even when the piperonyl butoxide was applied at 10.8 μg/fly.

RESULTS AND DISCUSSION

Female KUN flies treated with malathion at three concentrations (1.08, 0.54, and 0.27 μg/fly) showed that in all cases where apparent death occurred, there was an initial marked increase of oxygen consumption. (Figure 1.) Conversely, a marked increase of oxygen consumption was

never observed in cases where the treated flies survived. Each line in Figure 1 represents the average of several replicates.

In addition to the marked increase in oxygen consumption of malathion treated KUN females, several other changes were noted at the various concentrations. The first change was in relation to the average height of the oxygen consumption peaks. The average oxygen consumption of the flies receiving the highest dose (1.08 μg/fly) peaked at 114 μl./min./mg. of fly. At half the above dose the peak occurred at 89 μl./min./mg. of fly which is slightly above half the distance between the oxygen consumptions of untreated flies and those receiving the highest dose. The lowest dose (0.27 μg/fly) resulted in a slight rise in oxygen consumption.

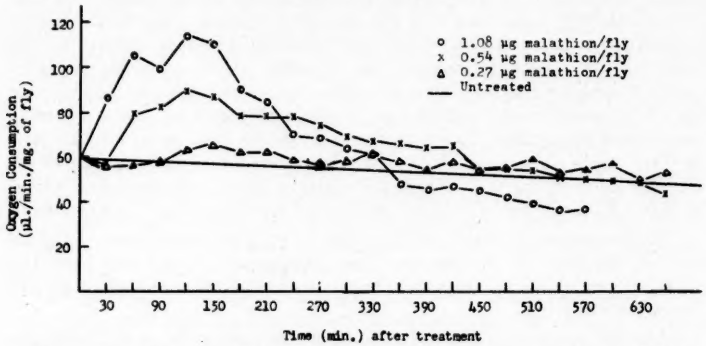

FIG. 1. The effect of malathion on the rate of oxygen consumption of malathion-susceptible female house flies (KUN).

The differences in the magnitude of the peak of oxygen consumption at the various dosage levels used were due primarily to the differences in the number of flies that eventually died. A sharp rise in oxygen consumption of a malathion-treated fly was always associated with death. At the highest dose, all 16 treated flies died. At the intermediate dosage 15 of 23 treated flies died. Only one out of 14 treated flies died at the lowest dose.

The second change noted was the latent period. The latent period is the time interval between treatment and the beginning of the sudden increase in oxygen consumption. Lord (1950) showed that this latent period was dependent on concentration (parathion on *Tribolium castaneum*), i.e., within a concentration range the duration of the latent period was inversely proportional to the concentration. Similar results were obtained in this investigation. Flies treated with 1.08 μg/fly of malathion showed a rise in oxygen consumption almost immediately. Half the above dosage caused a latent period of approximately 30 minutes before showing a sharp rise. The lowest concentration showed a latent period of 90 minutes (based on average of living and dying flies) while the detailed laboratory notes showed that the lone dead individual had a latent period of 60 minutes.

The third change noted was the time it took for oxygen consumption to drop to or below normal. It took 350 minutes (average of all dead) for

the highest concentration and 450 minutes (average of dead and alive flies) at the next lower concentration. At the lowest concentration oxygen consumption dropped to normal by 270 minutes and was erratically on or slightly above normal to the end of the experimental period.

Maximum rate of oxygen consumption in descending order for the three concentrations occurred at 120, 120, and 150 minutes after treatment. On an individual basis the maximum rate of oxygen consumption ranged from 60 to 220 minutes after treatment for the highest dosage level and 90 to 270 minutes for the next lower dosage level. Only one death occurred at the lowest dose and its peak oxygen consumption was at 150 minutes after treatment.

Topical application of malathion in combination with piperonyl butoxide on house flies results in antagonism. Rai, *et al.* (1956) and Ware (1957) showed that malathion was antagonized 1.60 times by piperonyl butoxide with a mixture of one part of the former with 10 parts of the latter by weight. The same ratio was used throughout this investigation. One dosage level contained a mixture of 1.08 micrograms of malathion and 10.8 micrograms of piperonyl butoxide per fly. The other dosage level was one half the concentration of above mixture.

The results (Figures 2 and 3) were based on an average of 23 flies for the higher dosage and 15 flies for the lower dosage. The maximum rate of oxygen consumption (80 μl./min./mg. of fly) occurred 300 minutes after treatment for higher dosage (on an individual basis the peaks ranged from 150 to 450 minutes), while at lower dosage level it occurred at 120 minutes (71 μl./min./mg. of fly). The difference in maximum rate of oxygen consumption was due primarily to the difference in ratio of dead to live flies. At the higher dosage 15 to 23 flies were dead while three of 15 were dead for the lower dosage. The latent period before a sharp rise in oxygen consumption was 150 minutes after treatment for the higher dosage and 90 minutes for the lower.

Original data showed that the three flies that died at the lower dosage level were sluggish and inactive before treatment. They would probably have survived had they not been in their weakend condition. Thus, the data of the lower dosage should not be given equal weight in evaluation.

Comparing the higher dosage of malathion plus piperonyl butoxide mixture with malathion alone (Figure 2), piperonyl butoxide greatly increased the latent period. The maximum rate of oxygen consumption with the malathion-piperonyl butoxide mixture occurred at 300 minutes after treatment. Ware (1957) showed that the rates of piperonyl butoxide penetration alone and in combination with malathion when topically applied to KUN males were generally identical. Unfortunately penetration studies of malathion alone were not conducted, thus the possibility that the delay in sudden rise of oxygen consumption having been due to interference of malathion penetration by piperonyl butoxide cannot be discounted.

Two dosage levels, 1.08 and 0.54 μg/fly, comparable to the doses used on KUN flies, were used on CAL females. There were no differences in oxygen consumption of the CAL strain at the two dosage levels. This was

expected since rise in oxygen consumption is directly correlated with death and no mortality was recorded at such a low level.

To further show the correlation of respiration and mortality, dosages of 1.08 μg/fly of malathion in combination with 10.8 μg/fly of piperonyl butoxide for one level and 0.54 μg/fly of malathion in combination with 5.4 μg/fly of piperonyl butoxide for another level were tested on the CAL females. No mortality or significant change in the oxygen consumption rate were observed at either level.

To compare two lethal concentrations on CAL females, dosages of 10.8 μg/fly and 5.4 μg/fly of malathion were tested. The results are presented in Figure 4. The higher dosage represents an average oxygen consumption of 15 females while the lower dosage represents an average oxygen consumption of 16 females. In both cases, all flies died.

The peak oxygen consumption of the higher dose was 110 μl./min/mg. of fly while the lower dose peaked at 94 μl./min./mg. of fly. The latent

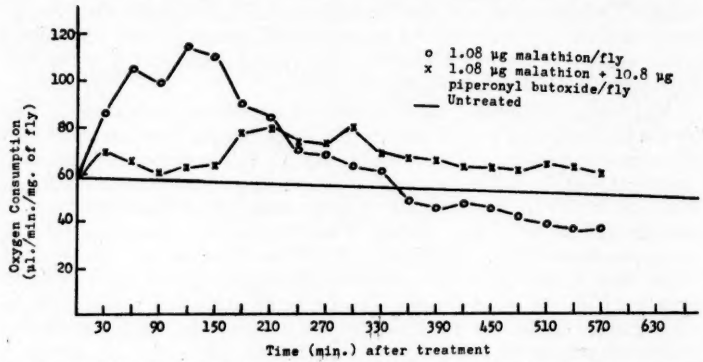

FIG. 2. Effect of piperonyl butoxide combinations on the rate of oxygen consumption of malathion-susceptible female house flies (KUN).

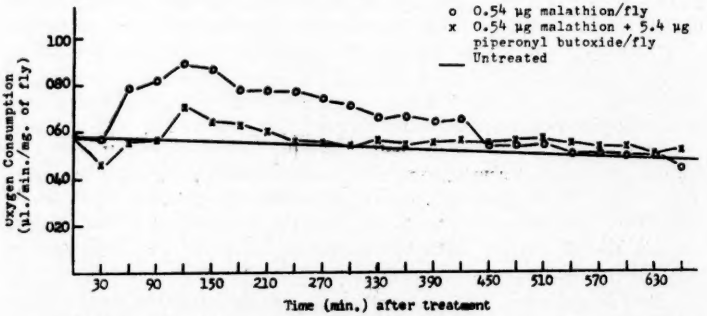

FIG. 3. Effect of malathion+piperonyl butoxide combinations on the rate of oxygen consumption of malathion-susceptible female house flies (KUN).

period was 60 minutes for the higher dosage and 90 minutes for the lower dosage. Both dosages caused a drop in oxygen consumption to the normal level at 420 minutes after treatment. The oxygen consumption reached a peak at 210 minutes for both dosages. On an individual basis, peaks of oxygen consumption ranged from 120 minutes to 330 minutes after treatment for the higher concentration and 150 minutes to 330 minutes for the lower dosage.

Figure 5 is a graphical comparison of the oxygen consumption of intoxicated CAL and KUN female flies. Both doses represent an approximate LC_{99}. KUN flies showed a peak of oxygen consumption at 120 minutes and CAL at 210 minutes; no latent period occurred with KUN flies, while CAL showed a latent period of about 60 minutes; and the oxygen consumption of KUN females dropped to normal at 350 minutes and of CAL females at 400 minutes.

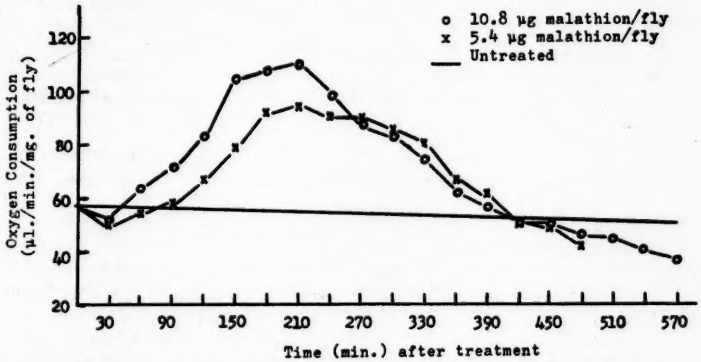

FIG. 4. Effect of malathion on the rate of oxygen consumption of malathion-resistant female house flies (CAL).

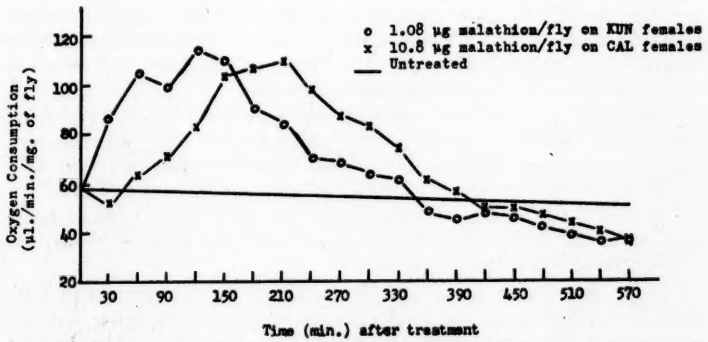

FIG. 5. Effect of malathion (approx. LD_{99}) on the rate of oxygen consumption of malathion-resistant (CAL) and -susceptible (KUN) female house flies.

The peaks of the oxygen consumption rates presented graphically were based on the average oxygen consumption of several flies. All flies did not have their rates of oxygen consumption peak at the same time. To be more accurate, the peaks must all be averaged regardless of time. Only dead flies should be considered since in cases where oxygen consumption of dying and surviving are averaged, the peaks of respiration of the dying flies are lowered by the surviving flies. Table 1 was calculated by considering the peaks of respiration of only dying flies regardless of time. The average peak was divided by the average rate of untreated flies (55 μl./min./mg. of fly) to give the magnitude of the increase in the rate of oxygen consumption.

TABLE 1. Degree of stimulation of oxygen consumption by malathion and malathion-piperonyl butoxide mixtures at a ratio of 1:10 on KUN (susceptible) and CAL (resistant) female house flies, *Musca domestica* L.

Dosages μg of malathion per fly	Degree of Stimulation[a]	
	Malathion	Malathion + Piperonyl Butoxide
	KUN flies	
1.08	2.6 times	2.1 times
0.54	2.1 times	2.4 times
	CAL flies	
10.8	2.4 times	
5.4	2.2 times	

[a] Calculated by dividing average of all rates of oxygen consumption peaks of dead flies by average oxygen consumption rate of normal flies, 55 μl/min./mg. of fly.

In both the CAL and KUN strains the degree of stimulation of oxygen consumption was more than two times, and reduced dosage lowered the degree of stimulation.

To follow the course of malathion intoxication, observations of treated flies were made before each manometric measurement. The relationship of oxygen consumption and symptoms are presented in Table 2. The maximum period between observations was 30 minutes.

The sequence of events manifested in intoxication was hyperactivity, knockdown, paralysis and ultimately apparent death. It was observed that flies undergoing paralysis always died. Only three flies recovered from knockdown, but these later exhibited paralysis and ultimately died.

The sharp rise in oxygen consumption rate of the treated female house fly was associated with paralysis. Flies not exhibiting paralysis showed no marked increase in oxygen consumption.

If the increase in respiration of the house fly were associated with oxidation of malathion, the sudden increase should occur before external symptoms of intoxication appear. Table 2 showed that the sudden increase in oxygen consumption did not appear before the first external symptom of

intoxication, but rather after the first symptoms. Further, the amount of oxygen required to oxidize malathion is very minute. Therefore, oxidation of malathion alone cannot explain the sudden increase.

In correlating symptomology and oxygen consumption, it was noted that a marked increase in oxygen consumption was associated with paralysis (Table 2). It would be expected that if the peak in the rate of oxygen consumption represents the period when most of the toxicant in its active form is or already has been at the site of action and further that cholinesterase depression is the primary cause of the sudden increase in oxygen consumption, then cholinesterase depression should be the greatest slightly before or during the period when the rate of oxygen consumption is highest.

TABLE 2. Relationship of symptoms and oxygen consumption in malathion-treated female house flies, *Musca domestica*, L.

Symptoms	Trend of oxygen consumption rate
No symptoms	Normal
Hyperactivity	Normal or slight increase
Knockdown	Normal or slight increase
Paralysis	Marked increase followed by slow return to normal or below
Apparent death	Below normal

Since cholinesterase activity was not tested in this investigation, a comparison of respiration is made with the results of Mengle and Casida (1958). Mengle and Casida used female flies treated topically with malathion on the ventral tip of the abdomen. They found that brain cholinesterase level was lowest during hyperactivity (20 minutes after treatment) and that when paralytic symptoms appeared, the cholinesterase level had partially recovered.

Thus, the rise in oxygen consumption cannot be directly correlated with cholinesterase depression. This suggests that there is a possibility that malathion affects other systems (in addition to cholinesterase) directly, or indirectly, resulting in the sudden increase in the rate of oxygen consumption.

It was hypothesized that since the rate of oxygen consumption reached a peak at a given time, mortality should be highest at a comparable time after treatment. This would be true if a sudden rise in oxygen consumption after treatment were correlated with mortality.

The following experiment was consequently conducted. Malathion (10.8 and 5.4 μg/fly) was topically applied to CAL females. Twenty-five flies were replicated eight times for the higher dosage and six times for the lower. Mortalities were recorded at intervals beginning at two hours after treatment. Moribund flies were considered as dead. One hundred flies were treated with acetone only and served as controls. No mortality was noted in the controls after 24 hours. The results are presented in Table 3.

Of 200 flies treated with 10.8 μg/fly of malathion, the maximum number of flies (27 per cent) died or were moribund between three and four

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Of 200 flies treated with 10.8 μg/fly of malathion, the maximum number of flies (27 per cent) died or were moribund between three and four

TABLE 3. Mortalities of female house flies, *Musca domestica* L.

Treated with 10.8 μg of malathion per fly.

Hours After Treatment	Number Dead 25 Flies per Replicate								Total Dead	Average % Mortality	Increased % Mortality Per Interval
	1	2	3	4	5	6	7	8			
2	2	2	2	4	2	3	2	2	19	9.5	9.5
3	6	5	6	9	6	4	5	5	46	23.0	13.5
4	14	13	14	14	13	9	11	12	100	50.0	27.0
5	19	17	17	18	16	13	14	18	132	66.0	16.0
6	24	24	20	20	22	20	17	22	169	84.5	18.5
24	25	25	25	25	25	25	25	25	200	100.0	15.5

Treated with 5.4 μg of malathion per fly.

Hours After Treatment	Number Dead 25 Flies per Replicate						Total Dead	Average % Mortality	Increased % Mortality Per Interval
	1	2	3	4	5	6			
2	5	3	2	1	3	3	17	11.3	11.3
3	5	10	6	5	6	9	41	27.3	16.0
4	10	12	12	8	12	13	67	44.7	17.4
5	15	14	14	9	16	15	83	55.3	10.6
6	16	18	16	11	18	18	97	64.7	9.4
7	19	20	18	16	19	19	111	74.0	9.3
10	19	24	24	22	23	22	134	89.3	15.3[a]
11	20	24	25	22	23	24	138	92.0	2.7

[a] 3-hour interval.

hours (Table 3, above). At the lower dosage (Table 3, below) the maximum number of flies (17.4 per cent) was apparently dead or moribund between three and four hours. Again this corresponds to the peak of oxygen consumption rate for CAL females (210 minutes) treated with comparable doses (Figure 3, below).

SUMMARY

Applications of malathion to female house flies increase the rate of oxygen consumption within one to two hours after treatment. House flies that are resistant to malathion do not show the increased oxygen consumption rate at the same doses, and at higher doses the peak occurs after a greater post-treatment time lapse. Joint applications of piperonyl butoxide and malathion reduce the magnitude of the oxygen consumption increase and shift the occurrence of this peak further from the time of treatment.

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AN UNUSUAL NEW SPECIES OF *BOREUS* (Mecoptera: Boreidae) FROM OREGON[1]

GEORGE W. BYERS

Most species of *Boreus* in North America—or throughout the holarctic range of the genus—are rather similar in their overall appearance, but an occasional species, such as *Boreus reductus* Carpenter, is conspicuously different from the others. Dr. John D. Lattin, of the Department of Entomology, Oregon State College, has recently sent me a few specimens repre-

[1] Contribution No. 1100 from the Department of Entomology, The University of Kansas.

senting such an unusual species. The following description is based upon one male and one female preserved in alcohol and two females pinned. Fortunately, the most conspicuous characteristic of the species is found in the female. I wish to express my thanks to Dr. Lattin for making these specimens available and for sending descriptions of certain of the collecting localities, also to Mr. Kenneth M. Fender, of McMinnville, Oregon, for collecting two of the specimens and for supplying me with descriptions of the habitats.

Boreus brevicaudus, new species

Head: Occiput, vertex and narrow area below eyes dark brown; rostrum in general, including mouthparts, yellowish brown. Eyes dark reddish purple, or plum colored; ocelli minute, widely spaced. Antennae with only eighteen segments, the scape broad, pedicel expanded in apical one third and subequal in length to first flagellar segment. Rostrum long in proportion to remainder of body. Coarse pubescence on vertex between eyes.

Thorax: Pronotum with two conspicuous transverse grooves, creating anterior and posterior ridges and a broad central area. Three large bristles on each side of anterior ridge, two on each side of posterior ridge. Modified wings in both sexes straw yellow in color, those in the male attaining only the third abdominal tergum, in specimen preserved in alcohol. Thoracic sclerites light brown, except for cervical sclerite, prothoracic pleura, and metepimeron, which are dark brown. Legs yellowish brown; tarsi darkening to brown at apex.

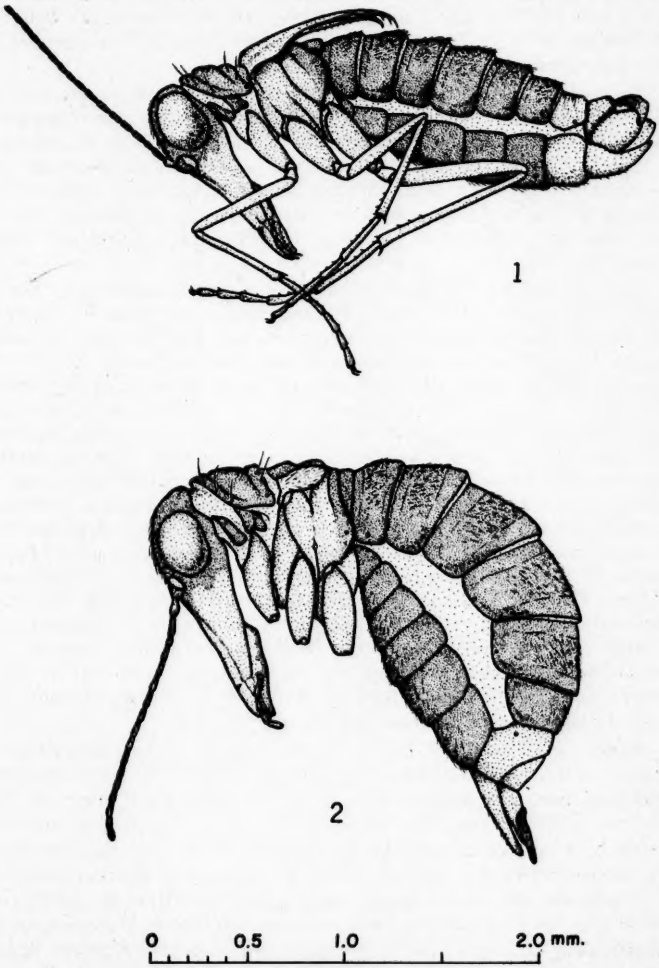
Abdomen: Abdominal terga and sterna of first seven segments, in both sexes, dark brown, with a metallic, greenish reflection in certain lights. Membranous areas paler brown. Eighth and subsequent segments yellowish to yellowish brown. Short pubescence chiefly in broad, transverse bands across terga, absent from anterior and posterior margins in female, from anterior margins in male; pubescence longer, less restricted in distribution on sterna. Spiracles situated in lateral margins of terga.

Ninth abdominal tergum of male (fig. 6) deeply cleft medially, not covering tips of fully retracted dististyles. About eight sharp-pointed, short, blackened spines or denticles on surfaces at either side of medial cleft. Tergum darker at margins than in middle area. Dististyles heavily sclerotized apically, paler basally, toothed and bearing an elongate inner appendage. Ninth sternum (hypandrium) broadly rounded at tip (fig. 7).

Ovipositor of female (figs. 3, 4, 5) very short, less than half length of rostrum. Lower valves completely separated. Cerci fused basally, separate apically but in close contact, heavily sclerotized, blackened except for extreme apex, each with a pale, oblong fenestra (thin, translucent portion of cuticle) at about mid-length. Tenth sternum produced caudally beneath eleventh (anal) segment in form of two strap-like extensions each bearing about three blackened teeth or short spines.

Measurements: Holotype male—body length 2.62 mm.; forewing 0.83 mm.; antenna 1.30 mm.; rostrum, eye to tip, 0.75 mm. Allotype female—body length 2.94 mm.; forewing 0.27 mm.; antenna 1.34 mm.; rostrum, eye to tip, 0.83 mm.; ovipositor, posterior edge of ninth tergum to tip, 0.38 mm.

Holotype: male, collected in Spencer Butte area, 2 miles south of Eugene, Lane County, Oregon, 27 November 1959, by D. Smith; the specimen was obtained from moss from a dead tree. *Allotype:* female, Spencer Butte, 3 miles south of Eugene, Lane County, Oregon, 23 November 1959, elevation 1400 feet, J. D. Lattin; from mosses and leaf litter, by Berlese funnel extrac-



FIGS. 1, 2. *Boreus brevicaudus*, new species. Fig. 1. Holotype, male, left lateral aspect. Fig. 2. Allotype, female, left lateral aspect, legs not drawn.

tion. Holotype and allotype are both preserved in alcohol and are in the collection of Oregon State College, Corvallis, Oregon. *Paratype:* female, Oneonta Gorge, Columbia River Highway, about 7 miles west of Bonneville, Multnomah County, Oregon, 16 October 1955, K. M. Fender; in the Snow Entomological Museum, The University of Kansas. This female seems to be teneral but is preserved dried, pinned. *Paratype:* female, Peavine Ridge, near McMinnville, Yamhill County, Oregon, 8 November 1946, K. M. Fender; in the collection of Oregon State College. This specimen is only fragmentary, lacking the head and thorax.

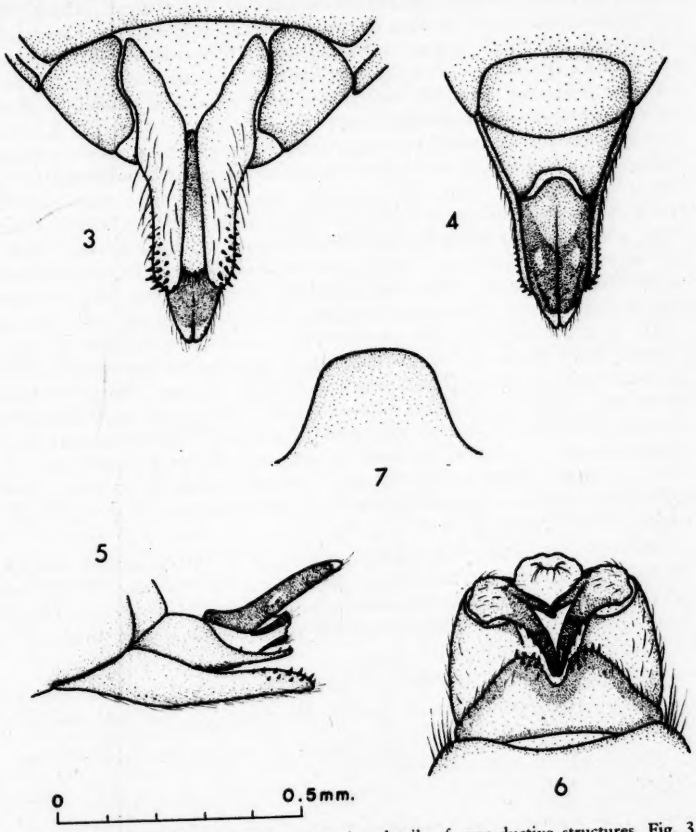
Discussion: Although both males and females of *Boreus brevicaudus* will be identified as *B. intermedius* Lloyd by use of existing keys (Carpenter, 1935, 1936), it has no closer relationship to that species than to any other in North America. In fact, the structure of the female ovipositor sets *brevicaudus* quite apart from all other known species of the genus. The ninth tergum of the male also is very distinctive and emphasizes the usefulness of this structure in taxonomy of *Boreus,* as pointed out earlier (Byers, 1954: 509).

In other species described as having a short ovipositor (see key to females, Carpenter, 1936: 61-62), the ovipositor is subequal in length to the rostrum, but by contrast that of *brevicaudus* is less than half the rostral length. The tenth segment in females of most species of *Boreus* has a nearly truncate apex, on which the fused cerci are borne; however, in *brevicaudus* this segment extends backward beneath the cerci and the concealed, mostly membranous eleventh segment (fig. 5) as two flattened, elongate processes, each bearing two blackened, short, stout spines near the apex and another near the base. Although these extensions, apparently of the tenth sternum, are similar to the lower valves of the ovipositor (which I regard as extensions of the eighth sternum) in texture, color and type of spines or denticles, their function in copulation—if any—is unknown. In all other species of *Boreus* known to me, the cerci are completely fused, forming the apparent eleventh tergum. Their identity is so obscure, in fact, that Hinton (1958: 204) states the cerci are lacking in *Boreus,* in which characteristic this genus is supposedly unlike the other Mecoptera. In *Boreus brevicaudus,* however, the somewhat thickened cerci are not wholly fused (fig. 4) and may be raised, revealing the true eleventh segment, which is largely membranous but bears dorsal and ventral sclerites (fig. 5).

Males of all species of *Boreus* I have examined have indentations, or sockets, in the ninth abdominal tergum, into which the tips of the dististyles may be drawn. In some species, such as *B. brumalis* Fitch, these are quite shallow, while in others (for example, *B. unicolor* Hine) they are deep, partly by reason of a hood-like development of the tergum above them. In *Boreus brevicaudus,* however, there are no such sockets, so that when the dististyles are drawn against the tergum, their tips are visible from above (fig. 6). Furthermore, there is no median lobe or projection in this species, such as is found between or above the sockets in the ninth tergum of other species (*cf.* Byers, 1954: 491; 1955: 3). Also, the spiniferous areas of the ninth tergum, which are more or less inclined toward one another

in nearctic species of comparable size (*brumalis* and *reductus*), are situated on divergent lobes in *brevicaudus*.

In most *Boreus* species, the antennae have twenty or more segments and far exceed the length of the head. Lestage (1941: 105) recorded 19 antennal segments in *B. chadzhi-gireji* Pliginsky and *B. aktijari* Pliginsky, Crimean species that were considered probably synonymous by Martynova (1954: 61). Another species, *B. vlasovi* Martynova (1954: 61), from the Turkmen and Tadzhik regions of the U.S.S.R., also has only 19 antennal segments.



FIGS. 3-7. *Boreus brevicaudus*, new species, details of reproductive structures. Fig. 3. Terminal abdominal segments and ovipositor of female (allotype), ventral aspect. Fig. 4. Ovipositor of female, dorsal aspect. Fig. 5. Ovipositor of female, left lateral aspect, with cerci elevated to show normally concealed eleventh segment. Fig. 6. Terminal abdominal segments of male, dorsal aspect. Fig. 7. Ninth sternum (hypandrium) of male, postero-ventral aspect.

In *Boreus brevicaudus,* the antennae have only 18 segments and are subequal in length to the head in the female and only a little longer than the head in the male. Furthermore, the eyes are somewhat smaller in proportion to the remainder of the head than in most species of the genus, and the antennal bases are not in front of the eyes but at the level of the lower edge of the eyes.

Geographical distribution: The elevations of the localities where the holotype and allotype were collected are respectively 1200 and 1400 feet, but that of Fender's collecting station on Peavine Ridge is only about 900 feet, and the bottom of Oneonta Gorge is less than 100 feet above sea level. Accordingly, *Boreus brevicaudus,* unlike most other western boreids, possibly is not a montane species. In fact, the four localities, lying in a broad arc about 150 miles in length, are rather closely associated with the valleys of the Willamette and Columbia rivers.

Ecological distribution: In a letter, Dr. Lattin has described the typical habitat as a steep hillside with conifers and some mixed broadleaved trees. The specimens were both obtained from mosses. My field notes (made during a summer collecting trip with Mr. Fender) describe the Peavine Ridge site as a small ravine wooded with Douglas fir, big-leaf maple, alder and Gary white oak, with an undergrowth of *Symphoricarpos,* bracken and wood ferns, small trees and broadleaved herbaceous plants. Much of the floor of the ravine is marshy with seepage from small springs. According to Mr. Fender, the specimen of *Boreus* came from a sample of moss gathered from beside one of these springs. He further reports that "the specimen from Oneonta Gorge was collected by shaking clumps of moss from the walls of the gorge on a 'beat square.' " The gorge is described as narrow, with "nearly vertical walls going up three hundred or more feet. Oneonta Creek . . . takes up most of the gorge floor, the remainder of which is rather coarse rock with some grasses, weeds, ferns and a few deciduous trees." Mosses and a few ferns grow sparsely on the generally barren walls of the gorge.

Seasonal distribution: All the known records are for November and the last half of October, but there are not sufficient data to show a peak of occurrence. It is not possible to predict whether there is also a spring period of emergence of adults, as the species has not been intensively sought.

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NOTES ON THE INDIAN SUCKING LICE *HOPLOPLEURA MANICULATA* (NEUMANN) AND *NEOHAEMATOPINUS ECHINATUS* (NEUMANN) (Anoplura: Hoplopleuridae)[1]

HARRY D. PRATT[2] AND CHESTER J. STOJANOVICH[3]

During his study of ectoparasites and arthropod-borne viruses in India, Dr. Harold Trapido of the Virus Research Centre, Poona, India, has sent the writers specimens of *Hoplopleura maniculata* (Neumann) and *Neohaematopinus echinatus* (Neumann). These were collected at Sagar, Shimoga District, Mysore, India, in December, 1958, from squirrels in the genus *Funambulus*. Since the original descriptions of Neumann and the later papers of Ferris dealing with these two species are now out of print and difficult for many entomologists to obtain, the two species are refigured in both sexes with short critical notes which may aid in the identification of these lice.

In 1909 the French entomologist Neumann published descriptions of two species of sucking lice, *Haematopinus (Polyplax) maniculatus* and *Haematopinus (Polyplax) echinatus,* from squirrels [*Funambulus* (as *Sciurus*) *palmarum*] collected at Rajkote, India. Neumann also recorded both species from specimens taken at Navapour, India, which were erroneously attributed to a bat, *Scotophilus wroughtoni.*

In 1921 Ferris transferred *maniculatus* to the genus *Hoplopleura* and figured the male and female based on specimens collected at Colombo, Ceylon, on *Funambulus tristriatus.* Some of these specimens are now in the U.S. National Museum. In 1951 Ferris and Stojanovich included *maniculata* in their key to the species of *Hoplopleura* of the world. No additional records as to distribution and hosts appears to have been published.

In 1912 Cummings transferred *echinatus* to the genus *Neohaematopinus.* Ferris (1923) described and figured what he thought was a male *echinatus* from Colombo, Ceylon. This specimen was made the type of a new species, *Neohaematopinus ceylonicus,* by Ferris in 1951, who had seen material of the true *echinatus* type from Agra, India, collected on *Funambulus.* No other records appear to have been published on this species.

Hoplopleura maniculata (Neumann)

Haematopinus (Polyplax) maniculatus Neumann, 1909, Archives de Parasitologie, 13: 521-523, figs. 21, 22.

Hoplopleura maniculata (Neumann) Ferris, 1921, Contributions Toward a Monograph of the Sucking Lice, Part 2: 112-113, figs. 71, 72.

Hoplopleura maniculata (Neumann) Ferris, 1951, The Sucking Lice, pp. 129, 183.

H. maniculata has acute angles on the posterior margins of the para-

[1] The Research published here was supported in part by Grant No. E-2117 of the National Institutes of Health, Institute of Allergy and Infectious Diseases.

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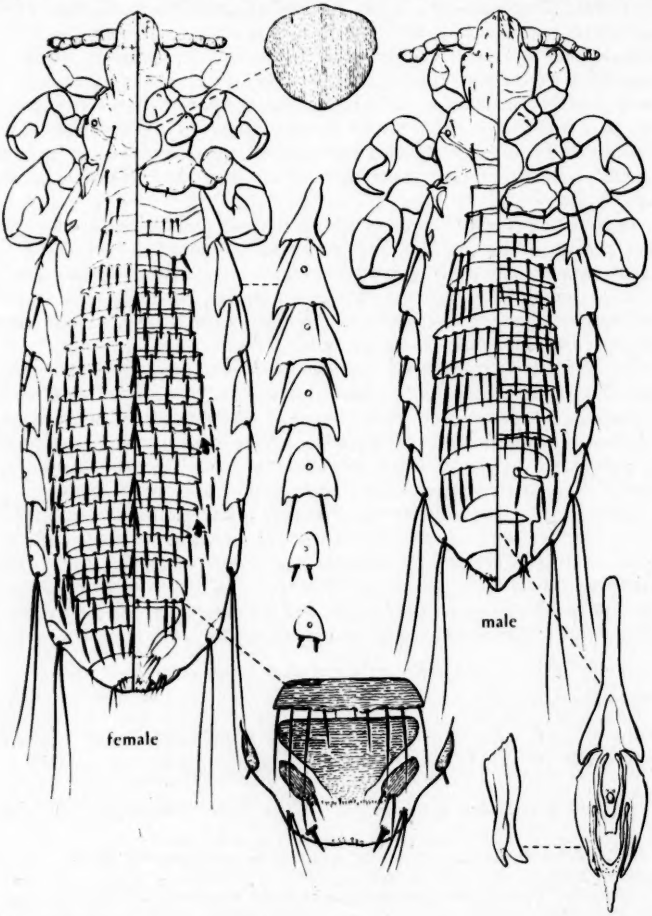
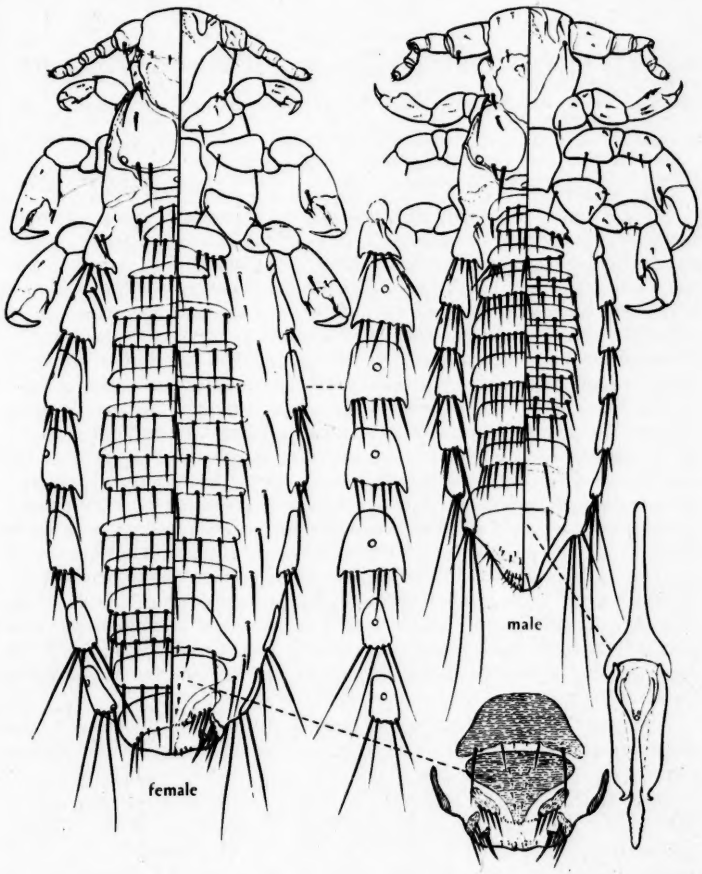
tergal plates on the third to sixth segments, similar in many respects to the Nearctic species *H. erratica* (Osborn) and *H. arboricola* Kellogg and Ferris found on chipmunks, *H. sciuricola* Ferris found on squirrels, or *H. hirsuta* Ferris found on cotton rats. It is closest to *erratica* and *arboricola* in that many of the specimens have the two stout spines on each side of the first sternite on segment III with the bases of these two spines set rather close together and placed on a projection. The majority of the abdominal setae are inflated, and the sternal plate of the thorax is rather short. *H. maniculata*



Hoplopleura maniculata (Neumann) Plate I

differs from *erratica* and *arboricola* in that the abdominal sternites and tergites are more strongly developed. None of the other species in this genus known to the writers, and none of the figures of *Hoplopleura* from all parts of the world figured by Ferris (1921), have a short seta on the ends of the last sternites on segments V and VI as shown by the arrows on Plate I. Ferris (1921) did not show these short setae in his drawings, figure 71, but they are present on female specimens of *maniculata* from Ceylon in the National Museum collection.



Neohaematopinus echinatus (Neumann) Plate II

The male genitalia are different from those of all Nearctic species except *arboricola* and *erratica* in having a definite notch at the tip of each paramere and in having a pronounced ear-like lobe on each side of the subgenital plate which bears a short and a moderately long seta.

Twenty-three male and 43 female specimens from Sagar, Shimoga District, Mysore, India, December, 1958, host *Funambulus* species, collected by Harold Trapido. Specimens are now in the collection of the Communicable Disease Center, U.S. National Museum, Virus Research Center, in Poona, India, and the private collection of Dr. K. C. Emerson.

Neohaematopinus echinatus (Neumann)

Haematopinus (Polyplax) echinatus Neumann, 1909, Archives de Parasitologie, Paris, 13:517, figs. 19, 20.
Neohaematopinus echinatus (Neumann), 1912, Cummings, Bull. Ent. Res., 3:393. (Generic transfer).
Neohaematopinus echinatus (Neumann), 1923, Ferris, Contributions toward a Monograph of the Sucking Lice, Part 3: 250-252, (female, not male).
Neohaematopinus echinatus (Neumann), 1951, Ferris, The Sucking Lice, pp. 189, 191.

N. echinatus apparently belongs in the *sciuropteri* group of *Neohaematopinus* which is characterized by a definite concave posterior margin on the sternal plate of the thorax, the male antenna being conspicuously more stout than that of the female, with the first segment markedly enlarged and bearing a stout spine, and the third segment with two strong, spine-like setae, and no rows of setae inserted in the membrane between abdominal sternites and tergites. *N. echinatus* differs from other species of *Neohaematopinus* in having a well-developed, rounded lobe bearing three or four stout setae at each end of the second sternite. This modification suggests the genus *Hoplopleura* which has a group of two or three stout spines on each side of the first sternal plate of the third segment of the abdomen; but, in *echinatus* this first sternal plate does not articulate with the adjacent paratergal plates as in the case of *Hoplopleura. N. echinatus* differs from the closely allied *N. ceylonicus,* both of which occur on squirrels of the genus *Funambulus,* in having two to four setae on the ventral lobe of paratergal plates III to VI, whereas in *ceylonicus* the ventral lobe of paratergal plates III to VI has only a single seta. In the single male of *echinatus* from Mysore collected by Dr. Trapido, the posterior portion of the male paramere is deeply emarginate before the tip, whereas Ferris' (1921, figure 161) drawing shows the paramere tapering gradually from the posterior fourth to the apex.

One male, and four female specimens with the same collection data as above. Specimens are now in the collections of the Communicable Disease Center, Virus Research Center, Poona, India, and U.S. National Museum.

Acknowledgments. The writers wish to acknowledge the assistance of Dr. Harold Trapido, Virus Research Centre, Poona, India, in obtaining the specimens; of Dr. Morris Tager, Department of Microbiology, Emory University and of Dr. D. S. Martin, Chief, Training Branch, Communicable Disease Center, for their continued interest in this research; and of Dr. K. C. Emerson, U.S. Army, for his constructive criticism of the manuscript.

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TWO NEW SPECIES OF *SEPSISOMA* FROM KANSAS (Diptera: Richardiidae)

GEORGE C. STEYSKAL
Grosse Ile, Michigan

In my recently published revision of the Richardiidae found north of Mexico,[1] only one species of *Sepsisoma* is listed. The following new species make the total three, distinguished as in the key below. In all three species the entire thorax is yellowish, while the other species of the genus from farther south have the thorax dark red-brown or black.

Key to the Species of *Sepsisoma* Johnson Found North of Mexico

1 (2). Humeral bristle lacking; anal cell markedly shorter posteriorly than anteriorly, anal crossvein distinctly retrorse; cell M_4 (3d posterior cell) at least 0.4 mm. wide; ocellar bristles distinctly posterior to anterior ocellus; hind tibiae dark brown to black ..... *S. flavescens* Johnson

2 (1). Humeral bristle present; anal cell approximately of equal length fore and aft, anal crossvein not retrorse; cell M_4 0.3 mm. or less in width; ocellar bristles in line with or slightly anterior to anterior ocellus; hind tibiae yellow to light brown. .....

3 (4). Length of wing 3.95-4.0 mm.; only minute hairs anterior to *dc* bristles; scutellar bristles less than half as far apart as *dc;* petiole of abdomen as in fig. 1a; male post-abdomen as in fig. 1 ..... *S. sabroskyi,* new species

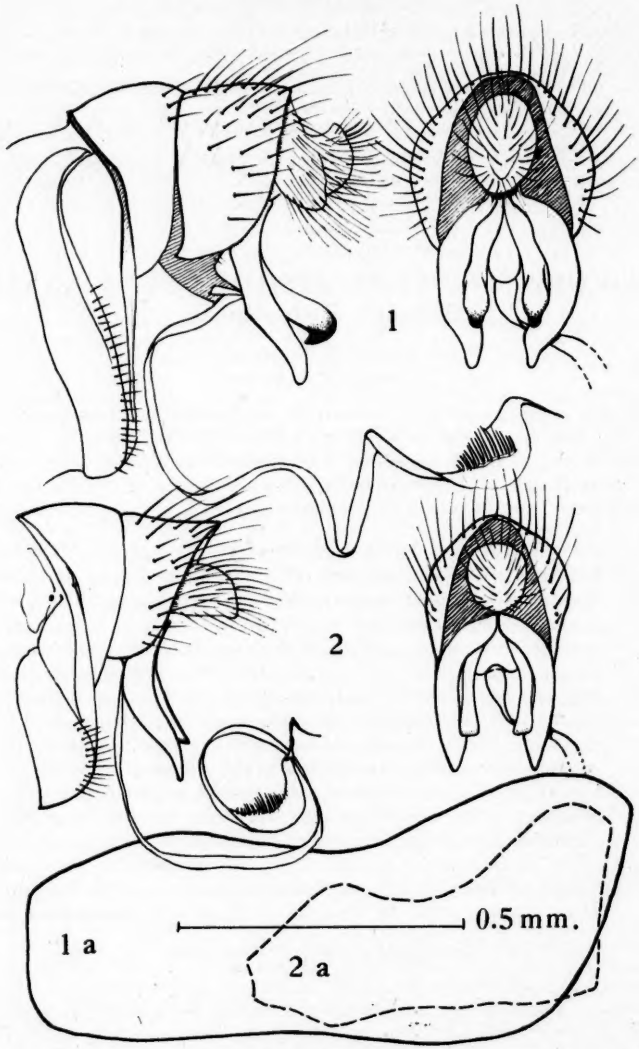
4 (3). Length of wing 3.0 mm.; a distinct although small second pair of *dc* present; *sc* 0.6 as far apart as *dc;* petiole of abdomen as

Sepsisoma sabroskyi, new species

(Figs. 1, 1a)

MALE. Length of wing, 3.95 mm. Color of body and legs wholly yellowish brown; bristles and hairs brownish to black; wings hyaline, with weak brown spot at apex; abdomen a little pitchy in middle.

[1] Steyskal, G. C. 1958. Notes on the Richardiidae, with a review of the species known to occur in the United States (Diptera, Acalyptratae). Ann. Ent. Soc. Amer. 51(5):302-310.



Sepsisoma sabroskyi, n. sp. Fig. 1, male postabdomen; Fig. 1a, profile of abdominal petiole.

Sepsisoma minimum, n. sp. Fig. 2, male postabdomen; Fig. 2a, profile of abdominal petiole.

Head somewhat longer and front flatter than in *S. flavescens;* ocellar bristles slightly anterad of anterior ocellus.

Thorax with dorsum subshining, pleura shining; one pair of strong *h;* one paid of *dc,* anterad of which are only minute hairs; one pair of apical *sc* 0.32 as far apart as *dc.*

Fore femora with three, hind femora with four very small subapical ventral spinules; middle femora lacking spinules.

Wings with anal cell approximately of equal length anteriorly and posteriorly; anal crossvein not retrorse, incompletely developed; free part of 6th vein very short; cell M_4 (3d posterior cell) 0.3 mm. wide; *ta* at 0.55 of length of discal cell (beyond middle); second vein extending to 0.8 of wing length.

Abdomen with profile of first tergite (syntergite, petiole) as in figure 1a; postabdomen as in figure 1.

FEMALE. Similar to male. Length of wing, 4.0 mm. Anal crossvein complete; *sc* bristles 0.37 as far apart as *dc.*

Holotype (male and allotype, Sand dunes, Medora County, Kansas, June 3, 1933 (C. W. Sabrosky), in United States National Museum.

Sepsisoma minimum, new species

(Figs. 2, 2a)

MALE. Length of wing 3.0 mm. Color of body and legs wholly yellowish brown; bristles and hairs brownish to b'ack; wings hyaline, with weak brown spot at apex; abdomen a little pitchy in middle.

Head somewhat longer and front flatter than in *S. flavescens;* ocellar bristles in line with anterior ocellus.

Thorax with dorsum subshining, pleura shining; one pair of strong *h;* one pair of *dc,* preceded by a distinct second pair about ¼ as long; one pair of apical *sc* 0.6 as far apart as *dc.*

Fore and hind femora each with two very small subapical ventral spinules; middle femora lacking spinules.

Wings with anal cell approximately of equal length anteriorly and posteriorly; anal crossvein complete, not retrorse; free part of 6th vein nearly half as long as anal cell; cell M_4 0.25 mm. wide; *ta* at 0.48 of length of discal cell (before middle); second vein extending to 0.8 of wing length.

Abdomen with profile of first tergite as in figure 2a; male postabdomen as in figure 2.

FEMALE. Unknown.

Holotype (male), Little Gobi, near Manhattan, Kansas, August 19, 1939 (C. W. Sabrosky), in United States National Museum.

Both of the above species are apparently more closely related to *Sepsisoma sepsioides* Schiner than to the generitype *S. flavescens* Johnson, differing from the former, according to Hendel's figure in Genera Insectorum, fasc. 113, fig. 57, in lack of black coloration and longer second vein.

I wish to thank the collector of these interesting flies, my friend Curtis W. Sabrosky, for the privilege of describing them.

NEW MOSQUITO RECORDS FROM NEBRASKA. I

WILLIAM F. RAPP, JR.
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Lincoln, Nebraska
and
FRED C. HARMSTON
U.S. Public Health Service
Greeley, Colorado

Since publication of Rapp's "Distributional Check-List of Nebraska Mosquitoes" (1959), several new records and some additional information have been obtained. Beadle (1959) published data on the human biting habits of mosquitoes at Mitchell, Scotts Bluff County, Nebraska. These data were obtained in studies conducted by the U.S. Public Health Service during 1953. Several mosquito records that were obtained from the studies at Mitchell, but not included in the article by Beadle, are incorporated in the present paper. Collectors of the various mosquitoes are found in parenthesis after each listing.

Anopheles Meigen

A. earlei Vargas: Mitchell, Scotts Bluff County, 2 females, light trap, 1953 (USPHS).

A. pseudopunctipennis franciscanus McCracken: Mitchell, Scotts Bluff County, 2 females, light trap, 1953 (USPHS).

A. walkeri Theobald: Reported to be the most abundant *Anopheles* at Mitchell, Scotts Bluff County, during 1953 (USPHS). Norfolk, Madison County, September 2, 1958, rare (Rapp).

Culiseta Felt

C. impatiens (Walker): Bayard, Morrill County, October, 1955, larvae, (Hopkins *et al.*, 1956:1472).

C. incidens (Thomson): Mitchell, Scotts Bluff County, 1 male, 1 female, light trap, 1953 (USPHS). Chadron, Dawes County, 1 male, light trap, July 30, 1957 (D. B. Gates).

Mansonia Blanchard

M. perturbans (Walker): Glen, Sioux County, July 27, 1959, 1 female, biting (Rapp-Harmston). Mitchell, Scotts Bluff County, "small numbers," 1953 (USPHS). Valentine, Cherry County, July 1, 1958, rare, light trap (Rapp).

Psorophora Robineau-Desvoidy

P. ferox (Humboldt): Homer, Dakota County, July 6, 1953, 2 females, biting (Rapp).

P. horrida (Dyar and Knab): Mitchell, Scotts Bluff County, 1 female, biting, 1953 (USPHS).

P. longipalpis Roth: Gates, Custer County, June 11, 1959, 1 female(Rapp-Harmston).

Aedes Meigen

A. c. canadensis Theobald: Victoria Springs State Park, Custer County, June 11, 1959, abundant and annoying in wooded areas (Rapp-Harmston).

A. flavescens (Müller): Antioch, Sheridan County, July 29, 1959, 1 female (Rapp-Harmston).

A. increpitus Dyar: Glen, Sioux County, July 27, 1959, 1 female, biting (Rapp-Harmston). This is the first record for this western species in Nebraska.

A. spencerii (Theobald): Bassett, Rock County, June 8, 1953, 2 females, biting (Rapp).

A. stimulans (Walker): Bassett, Rock County, June 8, 1953, 1 female, biting (Rapp). West Point, Cuming County, July 16, 1959, 1 female, biting (Rapp).

A. trivittatus (Coquillett): This appeared to be the most abundant *Aedes* mosquito in many areas in Nebraska during 1959 (Rapp).

A. cinereus Meigen: St. Paul, Howard County, June 9, 1959, 3 females (Rapp-Harmston). Gates, Custer County, June 11, 1959, 2 females, sweeping in grass (Rapp-Harmston).

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THE PENETRATION AND EXCRETION OF P^{32} LABELLED DIAZINON IN THE AMERICAN COCKROACH[1]

SAAD E. D. AFIFI[2] AND C. C. ROAN[3]

Diazinon, [0,0-diethyl, 0-(2-isopropyl-6-methyl-4-pyrimidinyl) thiophosphate] was first reported by Gysin (1952) as a potential insecticidal compound. This compound has also been investigated as a potential chemotherapeutic material. Its distribution and apparent metabolism in the American cockroach are reported here.

[1] Contribution No. 652, Department of Entomology, Kansas Agricultural Experiment Station. Work supported in part by funds provided by the U.S. Atomic Energy Commission under contract No. AT(11-1)-299.

[2] Presently with World Health Organization Malaria Team, Saudi Arabia.

[3] Professor of Entomology, Kansas State University, Manhattan, Kansas.

MATERIALS AND METHODS

Adult male American cockroaches, *Periplaneta americana* (L.), were used in these investigations. They were reared in the laboratory at a temperature of 23° C. and relative humidity of 50 per cent. All treatments and analyses were carried out at a temperature of 23° C.

The compound used in these investigations was P^{32} labelled Diazinon synthesized by Louloudes *et al.* (1956). Topical applications were made between the third and fourth abdominal tergites (under the wings). This site was selected to minimize external loss. Five microliters of an acetone solution of Diazinon were applied by means of a micropipette. Each cockroach received three micrograms of Diazinon. Five cockroaches were used for each test. Two replicates of each test were analyzed.

At the appropriate time intervals the cockroaches were anaesthetized with CO_2, removed from the holding jars, and rinsed thoroughly with acetone to determine the amounts of Diazinon remaining on the outside. The holding jars containing the fecal matter were also rinsed with acetone to determine the rate and quantities of excretion. The acetone solutions were concentrated to a constant volume prior to aliquoting for radiometric assay.

The cockroaches were dissected into the various components, the tissues pooled and wet-ashed with concentrated HNO_3. The acid solutions were made up to volume and a 0.2 ml. aliquot was pipetted into aluminum planchets for radiometric assay with a thin window GM tube (1.4 mg./cm^2) shielded with 2 inches of lead. A conventional decimal scaler was used.

The enzymatic determinations were made using the method of Hestrin (1949) as modified by Roan and Maeda (1954). A purified acetylcholine esterase obtained from Nutritional Biochemicals Corporation was used in these tests. When tissue extracts were used as sources of potential inhibitors, they were made to a 3 ml. volume and a 0.1 ml. aliquot was added to the enzyme preparation. When complete tissues were employed, the standard volume of enzyme solution was added to the tissue and brought to volume with buffer. The inhibitor and enzyme were always allowed to react for 15 minutes before the addition of the substrate. The buffer solution used for the enzyme tests and incubation of tissues with Diazinon was as follows: 0.15 M sodium chloride, 0.04 M magnesium chloride, and 0.025 M sodium bicarbonate.

The oral doses for the enzymatic studies were administered with micropipettes. In the experiments where the foregut was ligatured at its junction with the midgut, operative controls were held for at least 24 hours after sacrifice of the experimental cockroaches. No mortalities were noted in the operative controls.

PENETRATION AND DISTRIBUTION

The cockroaches were sacrificed and dissected at one-half, one, two, three, four, five, and six hours after treatment. Between the fifth and sixth hour most of the cockroaches were prostrate. Previous experience had shown

that prostration is not necessarily a sign of death and that the heart may, in fact, keep active for a considerable time after prostration.

The data for penetration and excretion are shown in Table 1. The applied dose had not penetrated completely by the time of prostration. The rate of penetration appears to be slower than that found in previous studies of several organic phosphorus compounds. Excretion starts somewhat more rapidly than in the case of tetraethyl pyrophosphate, para-oxon, and related compounds. This may be explained in part by the rapid concentration of a P^{32} labelled compound in the hindgut as shown in Table 1. The data in this table indicate an initial concentration of P^{32} in the foregut followed by a decrease after the third hour. There is a slow increase of labelled compound(s) in the midgut. The rapid increase in the hindgut is difficult to account for by way of the fore- and midgut, and indicates a possible action of the Malpighian tubules in excretion of Diazinon or its metabolites.

The foregut does not exhibit the rapid and selective concentration of P^{32} labelled compounds as was found in the case of tetraethyl pyrophosphate, para-oxon, etc. (Roan *et al.* 1950).

Two other tissues, the cuticle and fat body, were also analyzed. These data are also shown in Table 1. The tendency for P^{32} labelled compounds to concentrate in the fat body is of the same order as observed for the midgut. The cuticle shows a rate of build-up of P^{32} labelled compounds of the same order as the general penetration with a rather slow release into the rest of the body. Apparently Diazinon penetrates rather rapidly into the cuticle so as to be unavailable for external acetone extraction, but does not enter the circulatory system for rapid general distribution as was the case in the other organic phosphorus compounds investigated earlier.

In view of the indicated movement of a P^{32} moiety into and out of the foregut, oral doses were administered to cockroaches that had the foregut ligatured at its junction with the midgut. The ligaturing operation was not detrimental to the cockroaches, but the oral dose resulted in prostration within one-half hour after treatment.

TABLE 1. Penetration, distribution and excretion of P^{32} labelled Diazinon following topical application to the American cockroach.

	Per cent of applied dose					
Hours after treatment	1	2	3	4	5	6
Penetration	85	77	80	85	82	87
Cuticle	30	—	45	40	—	35
Fat body	7	8	4	5	3	8
Foregut	8	6	11	10	7	6
Midgut	2	5	3	6	4	7
Hindgut	4	20	18	17	30	29
Excretion	2	6	12	18	32	25

The tissues were removed prior to prostration and ground in the buffer used for the enzyme studies. One-tenth milliliter of this solution was added to the enzyme preparation as a potential inhibitor. These tests indicated the presence of an inhibitor in the macerate of the foregut.

The various tissues were then dissected from untreated animals. They were placed in the buffer with 350 micrograms of Diazinon and incubated for 50 minutes at 37° C. At the end of the incubation period, 0.1 ml. was removed and used as an inhibitor with the purified enzyme. The results of these studies are shown in Table 2. A very low level of inhibition results from the Diazinon alone. Only the foregut appears to produce any marked change in the antiesterase activity of the Diazinon or its metabolites.

The inability to extract from the various tissues exposed to Diazinon *in vivo* or *in vitro* any material with a potent antiesterase activity suggested the presence of a water soluble compound. To test this, the foregut of an American cockroach was dissected out. After clamping the foregut at the junction with the midgut, 350 micrograms of Diazinon in 0.1 ml. acetone was injected into the lumen of the foregut and the other end clamped off. The foregut containing the Diazinon was then placed in 3 ml. buffer and incubated for 50 minutes at 37° C.

At the end of the incubation period, the foregut was carefully removed from the buffer and placed in fresh buffer and ground in an all-glass homogenizer. One-tenth milliliter of the buffer used for the incubation and 0.1 ml. of the ground foregut-buffer mixture were added to enzyme preparations for determination of inhibitory activity. No inhibition was obtained from the ground tissue, but the aliquot from the first buffer solution produced 26 per cent inhibition. Assuming complete metabolism and diffusion of the metabolite from the gut, the aliquot taken represents a final concentration of 8.3×10^{-6} M in the enzyme test solution. The quantity of Diazinon alone required to produce this level of inhibtion is of the order of 2×10^{-4} M. These data suggest the metabolism of Diazinon in the lumen or cells of the foregut to form a water-soluble metabolite with considerably enhanced inhibitory activity.

TABLE 2. The inhibition of purified acetylcholine esterase by Diazinon after 50 minutes' incubation with various tissues of the American cockroach.

	350 μg. Diazinon plus					350 μg. Diazinon
	Foregut	Midgut	Hindgut	Fat body	Cuticle	
Per cent inhibition	24	7.5	0	0	0	3

SUMMARY

Phosphorus^{32} labelled Diazinon applied topically in acetone solution to the American cockroach appears to penetrate rather slowly. A considerable quantity is retained in the cuticle. The foregut shows an initial increase followed by a decrease. The midgut shows a steady increase as does the hindgut. The hindgut contains levels of P^{32} metabolites much higher than seems probable to account for by way of the midgut. The action of the hindgut or/and malpighian tubules in the excretion of P^{32} labelled metabolites is indicated.

The topically administered Diazinon is excreted rather rapidly as compared with other organic phosphorus insecticides previously investigated.

The foregut appears to metabolize Diazinon to a water-soluble metabolite with greatly enhanced anticholine esterase activity.

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——— and S. Maeda. 1954. *Ibid.*, 47: 507-14.

PIGMENTED BACTERIA OCCURRING IN FLY CULTURES AND SOME RELATIONSHIPS TO HOUSE FLY STRAINS[1]

JOHN L. ZAHARIS, C. C. ROAN, AND THOMAS H. LORD

The spontaneous appearance of a violet color in Cellucotton[2] watering pads placed in house fly, *Musca domestica* L., rearing cages was observed in October, 1958. This color appeared and spread over the surface of watering pads when a parathion-resistant strain of flies designated as CAL was present, or when flies were excluded from coming in contact with the watering pad. The color would not appear spontaneously nor would it spread after inoculation of new Cellucotton in a watering dish where an insecticide-susceptible strain of flies designated as KUN was present. Instead, watering pads routinely developed a butter-yellow color after about four days' exposure to the KUN flies. The work reported in this study is concerned with the isolation and classification of the pigmented bacteria present in the watering dishes, determination of some of the relationships of the bacteria to two fly strains, and determination of some factors involved in the selective appearance of the colors on the watering pads.

REVIEW OF LITERATURE

The association of bacteria and other invertebrates with house flies has been described in various publications. Perhaps the most comprehensive is that of Steinhaus (1946) who described approximately 60 species of bacteria directly associated with house flies.

Resistance of house flies to insecticides has been known since the late 1940's. A comprehensive review of the history of insecticide resistance in anthropods is given by Brown (1958).

[1] Contribution No. 774, Department of Entomology, and No. 361, Department of Bacteriology, Kansas Agricultural Experiment Station, Manhattan. A portion of a dissertation submitted by the senior author in partial fulfillment of the requirements for the degree Doctor of Philosophy, Kansas State University.

[2] An absorbent wood pulp product of the Kimberly-Clark Corporation.

In an attempt to find the underlying mechanism of resistance in insects, workers have described physiological, morphological, and behavioral differences between susceptible and resistant strains of a given insect species. Sactor (1950) reported consistently greater cytochrome oxidase activity in adult resistant flies than in susceptible ones and later related these findings to respiratory metabolism. Others have reported that resistant flies can detoxify 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT) to its ethylene derivative 2,2-bis-(p-chlorophenyl)-1, 1-dichloroethylene (DDE) more efficiently than susceptible flies (Lindquist *et al.*, 1951; March and Metcalf, 1950; Sternburg *et al.*, 1950; Winteringham *et al.*, 1951). Babers and Pratt (1950) found that the cholinesterase (ChE) activity of the head tissue of susceptible house flies was greater than that of resistant flies during the first five days of adult life. Bigelow and LeRoux (1954) noted differences in the width of the second abdominal sternum and the width of the frons in DDT-resistant and nonresistant strains of the house fly. They concluded that the differences were genetically associated with the factors producing DDT-resistance. Sokal and Hunter (1955) obtained results which contradicted the findings of Bigelow and LeRoux. Metcalf *et al.*, (1956) found an aromatic esterase capable of hydrolyzing both phenyl esters and organophosphorus esters in the tissues of normal house flies. Chadwick (1954) observed the 2,2-bis-(p-fluorophenyl)-1,1,1-trichloroethane (DFDT-)tolerant strain of flies at Edgewood was more placid in its behavior than the normal strain. And Missiroli *et al.*, (1948) found DDT-resistant flies south of Rome less active there than normal flies.

MATERIALS AND METHODS

An insecticide-susceptible strain of flies designated KUN was obtained from the University of Kansas in 1954. It has subsequently been maintained at Kansas State University with no insecticide pressure. A parathion-resistant strain of flies, CAL, was obtained from the Citrus Experiment Station laboratory at Riverside, Calif. It was approximately 75 times more resistant than a susceptible strain. To keep the level of resistance up, a parathion solution in a concentration of 0.025μg. per μl. of acetone was applied topically to the thoracic sternum of each two-day old fly in each generation, by Roan and Maeda's method (1953).

Ten-inch cubic fly cages were used to hold adult populations of house flies. The cages were made with screened tops and sides, a sliding glass panel for a front, and a rubber back with a hole, through which the hand could be inserted, covered with a jar lid three inches in diameter. Three-tenths ml. of fly eggs was planted in standard Chemical Specialties Manufacturers Association fly larva medium in earthenware crocks. This volume of eggs equalled 2000 eggs $\pm$ 180. After eight days, pupae were separated from the medium and placed in a No. 56-07-02, 7-ounce wax covered Dixie food dish. The dish with the pupae was placed in a clean cage together with a dish of water-saturated Cellucotton and a dish containing approximately three ounces of a 2-to-1 mixture of Starlac brand nonfat powdered milk and table sugar. This method was used for both strains studied. Other times the flies were fed on diluted Pet evaporated milk.

Cultures of both violet and yellow bacteria were maintained on Cellucotton pads in the fly rearing room prior to pure culture isolation on artificial media. Freshly prepared dishes were inoculated with a small piece of violet Cellucotton, and the pads were moistened daily with tap water applied with a polyethylene squeeze bottle. In three to six days, the violet color developed on the new pads. Yellow cultures on watering pads were obtained by sprinkling a small amount of a 2-1 mixture of Starlac-sugar on the surface of a fresh pad and allowing one to three days for development of the yellow color. These dishes were also moistened daily. The temperature in the fly room was maintained at 24.5°C. ± 2.5°.

Bacteria for species determinations were obtained from water expressed from Cellucotton watering pads exhibiting the violet and yellow colors. These specimens were streaked on starch agar plates and incubated at 20° to 25° C. for 48 hours. All apparently dissimilar colonies were restreaked for purification on starch agar plates and incubated as above. The colonies developing from the cultures were stained by the Hucker modification of the Gram stain and observed microscopically for purity. Apparently pure cultures were maintained on starch agar slants at 20° C. Colony characteristics on Petri plate cultures and on slants were recorded. Impure cultures failing to separate by the streak method were subcultured by the dilution method until pure and separate colony types were obtained. Organisms were identified using the methods recommended by the Society of American Bacteriologists (1957) and the 7th edition of Bergey's Manual of Determinative Bacteriology (Breed *et al.*, 1957). All media were prepared by the investigator according to the methods described by Lord (1959).

To maintain continuity between methods and results, they are reported consecutively below.

RESULTS AND DISCUSSION

TEST 1. Determining origin of violet color on Cellucotton. Pairs of 30 ml. beakers were set up as follows:

A. Sterile Cellucotton, sterile water, and cotton stoppers.

B. Sterile Cellucotton, sterile water, and screen tops.

C. Sterile Cellucotton, sterile water, and open tops.

D. Nonsterile Cellucotton, tap water, and cotton stoppers.

E. Nonsterile Cellucotton, tap water, and open tops.

F. Nonsterile Cellucotton, tap water, screen tops, and 4 three-day-old female KUN flies in one beaker, 4 CAL flies in the other beaker.

G. Sterile Cellucotton, sterile water, cotton stoppers, and 4 three-day-old female KUN flies in one beaker, 4 CAL flies in the other beaker.

All beakers containing sterile Cellucotton were kept moistened with sterile water. Cotton plugs were used to exclude everything except air. Screen tops of 14 mesh were used to keep out loose flies and cockroaches, both of which were cultured in the rearing room. Results of color development during the two-week test are shown in Table 1. Cotton plugs then were removed from the beakers in which color had not developed. Within

three days, violet color appeared on the autoclaved Cellucotton pads contained in the beakers.

TABLE 1. Violet color development in sterile and nonsterile Cellucotton under various incubating conditions.

Incubation condition	Sterile Cellucotton	Nonsterile Cellucotton
Open top on beaker	Violet	Violet
Screen top on beaker	No violet	Violet
Cotton plug on beaker	No violet	Violet
Screen top on beaker with flies	Not run	Violet
Cotton top on beaker with flies	No violet * Violet	No violet * Violet

* Color after cotton plug had been removed long enough to allow growth of any organisms introduced from room atmosphere.

It was concluded that the autoclaved Cellucotton was not altered in any way that might inhibit the appearance of the violet color. That violet color developed in open beakers of both sterile and nonsterile Cellucotton suggested that the violet might be directly related to either an agent in the tap water, an air-borne agent, or an insect-borne agent. However, a violet color developed in beakers in which sterile water, sterile Cellucotton, and no top existed. This removed tap water as a causative agent. Since no color developed in a cotton-stoppered beaker of sterile Cellucotton containing four flies, it appeared unlikely that flies were the vector of the causative agent. The violet color appeared, however, in screened beakers of nonsterile Cellucotton containing flies. Since tap water and flies were eliminated, the only new factors introduced were air-borne dust particles and, very likely, air-borne microorganisms.

TEST 2. Effect of fly strains on color development.

To determine the effect of fly strains on development of violet and yellow colors, two cages of newly sorted pupae, one containing KUN and one CAL strains, were set up. In both cages, fresh watering pads were inoculated with a piece of violet Cellucotton. Three days later, when the flies were almost two days old, a violet color was evident on three fourths of the surface of the CAL watering pad. The KUN pad had approximately one fourth of its surface colored yellow. At that point, the violet dish was changed to the KUN cage, and vice-versa. Four days later, when the flies were over 4 days old, the dishes were removed and examined.

The yellow dish from the KUN cage transposed to the CAL cage had no violet color development on the surface. The one-fourth part of surface area originally covered with yellow remained about the same. Closer examination of the pad revealed development of a violet color on the underside of the moist pad. The violet dish from the CAL cage transposed to the KUN cage developed a yellow color on that portion of the surface not originally colored violet. It appeared that the presence of KUN flies prohibited development of a violet color on Cellucotton.

TEST 3. Effect of fly regurgitant and excreta on violet chromogenesis.

Dry material deposited on the glass pane of fly cages was scraped off with a spatula and deposited on individual watering pads in a small, circular pile. Violet inoculum was also supplied. The dishes were incubated in a clean cage in the fly rearing room, in the absence of any insects. After five days, the deposit from the KUN flies had dissolved slightly and a yellow ring of bacterial growth had become visible around the mound of deposit. All violet color was absent from this ring but a distinct ring of violet color was evident around the immediate edge of the yellow ring.

In the CAL dish, a very slight yellow ring developed, neither so large nor so distinct as that in KUN, and the violet color was not so distinctly excluded from the area of the yellow ring. Violet color completely replaced the CAL yellow ring in time.

In a third control dish containing a small mound of acid washed sand instead of dry regurgitant-excreta material, no yellow color appeared. The violet color that developed approached the extreme edge of the sand sample. The presence of a physical barrier apparently did not inhibit violet color development. It appeared that a yellow bacterial growth, by priority, physically or chemically prevented violet color from developing on a given spot. Apparently the excreta and/or regurgitant provided a suitable substrate for development of the yellow pigmented organisms. Since the flies were fed either Starlac-sugar mixture or diluted Pet evaporated milk, one of those apparently was directly or indirectly responsible for the yellow color which appeared on Cellucotton watering pads.

TEST 4. Effect of sugar and powdered nonfat milk on yellow color development.

Two cages of KUN pupae were set up, one cage containing a food dish of granulated sugar as the only food source; the other, a food dish of dehydrated nonfat milk. Each cage contained a watering dish. Either granulated sugar or dehydrated milk as a food apparently produced the same effect on development of yellow on Cellucotton watering pads. That some of the food was carried over to the surface of the watering dish physically by the flies could not be ruled out. The presence of the yellow color on regurgitant and/or excreta obtained from fly cages indicated that this method alone could account for development of yellow organisms on Cellucotton.

TEST 5. Effect of change in food form on color development in KUN and CAL cages.

To eliminate dry foods as a determining factor in color development, a KUN and CAL cage, each with approximately 200 flies, were supplied with a Cellucotton pad saturated with 50 per cent diluted evaporated milk. Watering dishes were inoculated with violet Cellucotton plugs and examined six days later for color development. The pad from the KUN cage exhibited a drab-grey color, with the violet color visible on the underside of the pad. On the other hand, the pad from the CAL cage was colored violet over the entire surface and approximately one-half inch along the sides, where the

pad separated from the dish wall. The population of CAL flies did not inhibit the violet color.

TEST 6. Effect of sex of KUN flies on color development.

Four cages were set up containing watering dishes with inoculum. Two cages contained dry Starlac-sugar as food, and the other two, diluted evaporated milk. Two hundred male and two hundred female adult flies, four and five days old, were placed by sex in each cage of a pair. On the fourth day, some violet color developed in the watering pad of the cages containing male flies fed Starlac-sugar and the one containing males fed evaporated milk, but many of the males were dead in the cage containing evaporated milk, probably from old age. No violet color appeared in the cages of female flies. On the fifth day, very limited violet color appeared around the plugs in the watering pads of both cages containing females. Some yellow appeared on the surface of the pad in the cage of females fed Starlac-sugar.

TEST 7. Effect of numbers of KUN flies on inhibition of violet color.

To eliminate the possibility that the appearance of a violet color in the cages of flies (male or female) was due to numbers rather than sex, KUN pupae were placed in cages in lots of 100, 350, and 500. Inoculated watering dishes and a pad of Cellucotton saturated with sugar solution were supplied in each cage. Three days after flies emerged, violet color appeared on the surface of the watering pad in the cage of 100 flies. No color developed on the watering pads of either of the other two cages. It was concluded that between 200 and 350 adult flies were necessary to inhibit the violet color.

TEST 8. Development of violet color from agar culture.

A mixed culture growing on starch agar, but exhibiting some violet color was used to inoculate a 250 ml. Erlenmeyer flask containing sterile Cellucotton, and stoppered with a cotton plug. The Cellucotton was moistened only once with sterile water and the flask was incubated in the fly rearing room. Because the cotton stopper retarded evaporation considerably, it was 29 days after the test flask was inoculated before a violet color appeared on the surface of the sterile Cellucotton.

TEST 9. Identification of the yellow organisms in KUN cages.

From a dish of Cellucotton exhibiting a yellow surface color on the watering pad, a flamed Nichrome wire loop was used to isolate bacteria that could grow aerobically on starch agar. A loop of material from the surface of the yellow Cellucotton was inoculated into 5 ml. of sterile water in a test tube. After shaking the tube by hand approximately 5 minutes, a loopful of the suspension was streaked onto Petri plates of starch agar. Incubation was at approximately 25° C. unless testing required other temperatures. Identification was based upon morphological characteristics and biochemical reactions. The former included size, shape, and structure of the cells, form, size, color and appearance of individual colonies and agar slant streaks. The biochemical reactions included carbohydrate utilization, litmus milk reactions, starch, gelatin, and urea hydrolysis, catalase production,

nitrate reduction, atmospheric carbon and nitrogen utilization, relationship to free oxygen, indole production, and IMViC reactions.

The yellow organisms were identified as *Sarcina flava* de Bary, 1887, and *Flavobacterium breve* Bergey *et al.*, 1923. In addition, four apparently dissimilar colorless types were isolated from the yellow dish, but were not identified.

TEST 10. Identification of the bacteria associated with the violet color on Cellucotton.

Isolating the organisms from a dish of violet-colored Cellucotton proved to develop chromogenesis when streaked on starch agar. A colorless, highly mucoid, fast growing, gram-negative bacillus always developed in association with any isolates from the violet Cellucotton. The organisms responsible for the violet color on Cellucotton produced a rusty-brown color on starch agar. Furthermore, this color developed only when the culture became highly mucoid. The color became visible as an opaqueness in the mucoid culture. This opaqueness became faint blue, changing slowly to violet and then rusty-grown. The mucoid growth covered the pigmented area. This colored growth became a pellicle which could be lifted off the agar with a wire loop. Microscopic examination of the unstained pellicle revealed a mass of nonmotile, rod-shaped cells.

Separation of colonies could be obtained on starch agar by taking a loop of an agar culture early in development, inoculating 5 ml. of sterile distilled water in a test tube, shaking the tube by hand for 5 minutes or more, and streaking a loopful of the suspension on two plates in succession without flaming the wire between the first and second plate. Although some colonies still produced the highly mucoid growth, generally good isolation of smooth, colorless colonies was obtained on the second plate in the series. Three colony forms were obtained in every attempt at isolation. The first was a circular, convex, smooth, glistening, entire, opaque, homogeneous, colorless colony. The second was a circular, convex, smooth, entire, translucent, amorphous, yellow colony. The third colony form was a mixture of these two. Where the yellow and colorless colonies were contiguous, a faint blue iridescence was visible under reflected light, but no color of a true violet or rusty-brown nature ever became visible—unless the colorless colony form was of the mucoid type. Where the original drop of water suspension was placed on the first plate, the mucoid form of the colorless colony always developed, eventually producing the rusty-brown color characteristic of first isolations from a violet Cellucotton pad.

When the water suspension was transferred to a nutrient broth tube and incubated at 25° C. from one to twenty-four hours, all samples streaked out on starch agar from the broth produced only the colorless and yellow colonies, never the colorless mucoid form with resultant violet to rusty-brown chromogenesis. Inoculation of the colorless and yellow organisms into flasks of sterile Cellucotton produced no violet color. Inoculation with a water suspension of violet culture from starch agar produced the violet color on sterile Cellucottton. The colorless organism was highly pleomorphic. Because the mucoid form is obtained from water suspensions, it

is believed to be responsible for violet Cellucotton in conjunction with the yellow organism. The explanation for this phenomenon lies in the realm of speculation at present. Some physico-chemical change takes place which induces pigmentation in certain areas.

Evidence that the rusty-brown growth on agar is really responsible for violet color on Cellucotton comes from an indirect visual observation made in the course of testing. A culture of very dark pigmented *Chromobacterium violaceum* Bergonzini, 1881, became blue when placed on a saturated pad of Cellucotton. This lightening of the color suggests how the rusty-brown color on starch agar could look violet on Cellucotton.

DESCRIPTION: *Pseudomonas salopia* Gray and Thornton, 1928.

The colorless organism was a pleomorphic, aerobic, heterotrophic, gram-negative bacillus averaging 0.5μ by 1.5 to 1.75μ; motile, with monotrichous flagellation; nonacid fast; without a capsule; granules present; litmus milk peptonized on surface without any visible curd formation or pH reaction, but slight reduction of litmus at bottom of the tube; glucose weakly acid within 48 hours without gas formation, and lactose showing acid only after 5 days without gas; starch and Frazier's gelatin agar not hydrolyzed; nitrates not reduced; catalase negative; optimum growth temperature 28° C., growing also at 20° C. and 42° C.; indole not produced; acetyl methyl carbinol not produced; methyl red test negative; Krumwiede triple sugar agar showed no change; no growth in Koser's citrate medium; growth in 5 per cent NaCl broth but not in 6 per cent NaCl broth; cellulose not digested. Insoluble violet pigment produced, localized markedly.

DESCRIPTION: *Flavobacterium lutescens* (Migula, 1900) Bergey *et al.*, 1923.

The yellow organism was an aerobic, heterotrophic, gram-negative bacillus measuring 0.5μ by 0.95 to 1.5μ; nonmotile; nonacid fast; capsule absent; granules absent; litmus milk peptonized completely within 5 days without visible curd formation or pH reaction, but with some reduction of litmus with visible yellow growth at bottom of tube; glucose and lactose broth becoming alkaline within 3 days; Frazier's gelatin agar hydrolyzed within 48 hours with yellow growth; starch not hydrolyzed; nitrates reduced to nitrites; catalase positive; indole not produced; acetyl methyl carbinol not produced; methyl red test negative; Krumwiede triple sugar agar showed utilization of dextrose; no growth in Koser's citrate medium; growth in 5 per cent NaCl broth but not in 6 per cent NaCl broth; cellulose not digested; abundant growth on potato.

CONCLUSIONS AND SUMMARY

The obvious difference observed in the appearance of a violet color on Cellucotton in the CAL fly strain cages or in the absence of flies compared with its complete absence on the surface of the watering pads in KUN fly strain cages suggests that the insecticide-susceptible strain is able to inhibit either the agent responsible for development of the color or the process of color development.

In 1926, Duncan discovered a bactericidal principle in eight species of arthropods, including *M. domestica* L. This substance was present in the gut content of all forms studied, and in the feces of insects. He determined that there appeared to be but one active principle involved in each species of arthropod, but that different bacteria manifested differing degrees of susceptibility to it. The authors think that the same principle is involved here in prohibiting violet chromogenesis. The bacterium responsible for the violet pigmentation on Cellucotton is sensitive to the bactericidal substance when present in sufficient quantity, as when 200 flies are present in a cage containing only one watering dish. The ring of inhibition described for Test 4 is similar to results of routine tests performed with antibiotic discs in bacteriology laboratories. This bactericidal principle apparently has little effect on yellow pigmented bacteria.

In previous examinations of yellow colored watering pads from KUN cages, it was twice noted that disturbing the surface of the pads to expose fresh Cellucotton beneath the growth resulted in violet color developing within 30 hours. This took place in dishes not exposed to flies during the 30 hours. Disturbing the surface to expose fresh Cellucotton is believed to have resulted in removal of the bactericidal substance previously deposited on the surface by the flies during the period of development of yellow pigmented bacteria. Another factor is believed to play a part in violet color development. It is oxygen tension. Since violet color appears only on the surface of any Cellucotton pad, and never in the center of any mass except when entrapped air has resulted from folding of Cellucotton layers in forming watering pads, this would, in part, explain why the violet color became visible when the surface of a yellow watering pad was disturbed, exposing fresh Cellucotton to the atmosphere.

The bactericidal principle may have been lost in the CAL fly strain as a result of selection for resistance by means of insecticidal pressure. The loss may have resulted from some change in the cellular metabolism of the cells lining the stomach of the fly. In describing Duncan's work, West (1951) stated that "it is rather definitely established that this substance is formed in the stomach, but it is not known whether this is by secretion of the gastric cells or whether it results from the process of digestion." Since it is unlikely that the process of digestion has been altered between the KUN and CAL strains, both of which were fed the same foods, it is conceivable that a change in cellular metabolism, as a result of the drastic effect of an organophosphorus chemical, brought about the loss of the bactericidal principle in CAL flies.

The conclusion drawn from this investigation may be projected to a public health application in light of its potential importance. If the bactericidal principle should normally be an effective natural controlling agent against any medically important microorganisms associated with house flies in nature, this natural control would be lost in some cases when resistant flies are produced in nature. Besides creating insects more resistant to insecticides, it is conceivable that a more efficient disease vector may be produced as a result of man's feeble attempts to control house flies in their natural habitat.

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CORRECTION

Acarologists will please note the following corrections in collection data associated with the description of *Archemyobia trinidadensis* Tibbetts, Jour. Kansas Ent. Soc., 30(4):147, 1957.

Host: *Caluromys trinitatis* (Trinidad Wooly Opossum). Locality: St. Patrick Estate, Arima Valley, Trinidad, 25 July 1953. Collector: W. G. Downs.

As given originally and incorrectly, the data read:

Host: *Philander trinitatis* (Trinidad Wooly Opossum). Locality: St. Patrick's Arima, Port-of-Spain, Trinidad, 25 July, 1953. Collector T. H. G. Aitken.

FLUCTUATIONS IN RESPONSE OF MALE, KUN-STRAIN HOUSE FLIES TO TOPICALLY-APPLIED MALATHION[1]

GARY F. KRAUSE AND CLIFFORD C. ROAN[2]

Variation in responses of insects to any particular stimulus is well known by biologists. Conditions affecting the sensitivity of individual test insects have been reported by several workers (Rai *et al.* 1956; Sun & Sun, 1953; Yamasaki & Ishii, 1957).

The relative sensitivity to treatment of an entire test group may fluctuate widely. Turner & Bliss (1953) found a 10-fold difference in the LD_{50} for pyrethrin with *Tribolium* over a period of two weeks. Using rotenone against the black chrysanthemum aphis, Potter (1949) observed a 6-fold change of the LD_{50} over a period of six years of experimentation. Gunther *et al.* (1958), reporting studies of toxicities of 44 compounds related to diphenylmethane, state: "Agreement among replications in a given series of tests was excellent, but variations among the averages of series repeated at different times were considerable and amounted to as much as 600% in the LT_{50} values and 1000% in the LD_{50} values." The confused flour beetle, (*Tribolium confusum* Duv.), was used as the biological indicator.

Such large fluctuations in sensitivity among test insects prompted an experiment designed to study the variation in LD_{50} estimates for topically-applied malathion to male, KUN-strain house flies (*Musca domestica* L.).

METHODS

Malathion[3] (99.6% pure) was diluted with glass distilled acetone to the following levels (micrograms per microliter acetone): 0.40; 0.35; 0.30; 0.25; 0.20; 0. The insecticide was applied topically with a micro-device designed by Roan & Maeda (1953).

Male, KUN-strain house flies, reared according to Chemical Specialties Manufacturers Association (CSMA) procedures, with slight modifications described by Rai *et al.* (1956), were used in the tests. The house flies in each batch (group of house flies differing in age by one week from another group) were four days old when the insecticide was applied. Batches were treated at weekly intervals during the experiment.

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[3] Malathion, technical grade and 99.6% pure, was supplied by the American Cyanamid Company, Stamford, Connecticut.

A randomized complete block design was used with batches as blocks. Within each batch six trials of 50 flies each were conducted for the five dilutions and control.

Response of the house fly to the insecticide was measured by counting dead (no movement of any appendage) flies 24 hours after treatment. Each observation represented the percentage of dead flies per trial.

RESULTS AND DISCUSSION

A probit analysis (Finney 1952) was performed for each of the six "within-batch" assays. Batch estimates of the LD_{50} were calculated by pooling the results from 300 flies representing each of six daily assays. A single estimate (LD'_{50}) based on the 10,800 flies in this study was calculated as a basis for a comparison of the within-batch estimates. Batch estimates, and an expression of these relative to the LD'_{50}, are given in Table 1. The batch estimates range from 55% to 117% of the LD'_{50}.

TABLE 1. Batch estimates of the LD_{50} individually, and relative to the over-all LD'_{50}.

Batch (Week)	Batch LD_{50} Estimate	Batch Estimate, as Per cent of LD'_{50}
1	24.83	117.18
2	11.63	54.88
3	21.08	99.48
4	23.00	108.54
5	22.15	104.53
6	16.14	76.17

An analysis of variance of the single-assay estimated LD_{50}'s was calculated to study the batch-to-batch effect. The complete analysis of variance is shown in Table 2. An F test indicated that batch sensitivities were unequal ($P<.001$). A regression analysis of batch LD_{50}'s indicated that they were essentially randomly determined. The corresponding intrabatch correlation was found to be .7805 which emphasizes the low variation of LD_{50} estimates within batches.

TABLE 2. Analysis of variance of single assay estimates of LD_{50}.

Source of variation	D/F	Mean Square	F	Probability of larger F
Among Batches	5	23.90	22.33	.01
linear	1	45.03	42.08	.01
quadratic	1	25.76	24.07	.01
cubic	1	19.72	18.43	.01
quartic	1	4.65	4.35	.05
remainder	1	24.55	22.94	.01
Within Batches	30	1.07		

Often knowledge of the variance of the distribution of the mean response at a level of malathion other than the LD_{50} may be desirable. An average percentage mortality (p) was found for each dosage level, and a one-way analysis of variance was calculated for each level. Estimates of the within-batch variance (σ^2_e) were obtained from the component of variation. Estimates of this variance component are given in Table 3.

TABLE 3. Estimates of within-batch variance obtained from binomial theory and from the analysis of variance.

Average Percentage Mortality	$\frac{p(1-p)}{n}$	Estimate of σ^2_e
93	17.02	21.53
88	29.10	28.89
77	53.96	62.54
62	65.26	46.02
35	62.82	59.02

Quantal responses are known to follow the binomial distribution; so that, aside from possible error in estimating P, the binomial variance (σ^2) can be estimated by $\frac{p(100-p)}{n}$. W. G. Cochran (1952) gave a test to compare an observed variance with the theoretical binomial variance. He used the following despersion test:

$$x^2 = \frac{n\sum_{i=1}^{b} (X_i - \bar{x})^2}{b\bar{x}(n-\bar{x})} \qquad [1]$$

when n is the number of insects per trial, X_1 the number responding per trial, b the number of trials and $\bar{x}$ the mean number responding within each batch.

Chi-squares resulting from [1] indicate that the within-batch variation is a satisfactory estimate of the binomial variance $\left(\frac{pq}{n}\right)$, when p is estimated from the data. Therefore it is assumed that the within-batch trials at any level constitute a series of binomial trials with the characteristic binomial mean and variance. The binomial variance cannot be minimized beyond $\frac{pq}{n}$; since this is the average or expected variance.

A particular series in which the probability of an event is changing from group to group but constant within a group is called a Lexis series. Kendall (1946) gave a test for the Lexis ratio:

$$L = \sigma \Big/ \sqrt{\frac{PQ}{N}}, \qquad [2]$$

which is the ratio of the observed standard deviation to the theoretical binominal standard deviation. It follows that if a Lexis series exists, the ratio [2] will be greater than 1. Kendall's test applied to the observations obtained in this study of house flies indicated that the hypothesis that $L=1$ should be rejected ($P<.005$). Since all sample L's were significantly greater than 1, the Lexis series was assumed to describe the response of the male house flies used in this study. Hence the problem of estimating response of the male house fly is immediately a problem of measuring the response of several batches. Whether the objective of the experiment is to estimate the LD_{50} or mean response at any level, the among-batch variation dictates sample size. The number of repetitions within a batch is determined by the binomial variation, $\frac{pq}{n}$, since the Lexis series is assumed.

SUMMARY

KUN-strain, male, 4-day-old house flies (*Musca domestica* L.) reared according to CSMA (Chemical Specialties Manufacturers Association) procedures, were used to assay malathion at several levels. Each bioassay was conducted between June 18, 1958, and July 23, 1958.

Several statistical methods were used to study the fluctuations in batch sensitivity over the test period. An F test of calculated LD_{50} estimates indicated significant among-batch variation. A dispersion test indicated that the within-batch variation at any level of malathion was an acceptable estimate of the binomial variance. The Lexis series was assumed, after a Chi-square test, to describe the response of the house fly, when several batches were studied.

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CONTENTS OF THIS NUMBER

Revised Classification of the Bumblebees—A Synopsis (Hymenoptera: Apidae)
H. E. Milliron 49

A Strange *Hydrometra* from the Malay Peninsula (Heteroptera: Hydrometridae)
Herbert B. Hungerford and Ryuichi Matsuda 61

Effects of Malathion and Piperonyl Butoxide Combinations on the Rate of Oxygen Consumption of House Flies
M. T. Ouye, Lallan Rai, and C. C. Roan 64

An Unusual New Species of *Boreus* (Mecoptera: Boreidae) from Oregon
George W. Byers 73

Notes on the Indian Sucking Lice *Hoplopleura maniculata* (Neumann) and *Haematopinus echinatus* (Neumann) (Anopleura: Hoplopleuridae)
Harry D. Pratt and Chester J. Stojanovich 79

Two New Species of *Sepsisoma* from Kansas (Diptera: Richardiidae)
George C. Steyskal 83

New Mosquito Records from Nebraska, I
William F. Rapp, Jr. and Fred C. Harmston 86

The Penetration and Excretion of P^{32} Labelled Diazinon in the American Cockroach
Saad E. D. Afifi and C. C. Roan 87

Pigmented Bacteria Occurring in Fly Cultures and some Relationships to House Fly Strains
John L. Zaharis, C. C. Roan, and Thomas H. Lord 91

Correction *re Archemyobia trinidadensis* (Acarina: Myobiidae) 100

Fluctuations in Response of Male, KUN-Strain House Flies to Topically-Applied Malathion
Gary F. Krause and Clifford C. Roan 101